The Composition and Mechanism of Formation of Gastric Acid Secretion

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ONE OF THE MOST IMPORTANT PROBLEMS confronting the clinical physiologist today is how the stomach makes its hydrochloric acid. Nowhere else in the body does a physiological fluid regularly attain a pH less than 3.0, let alone a value between 0.8 and 0.9 of a pH unit. Formation of such a solution requires the liberation of a strong acid, at concentration of about 0.17 N. This process has always fascinated me because of the simplicity of the chemical entity involved, but my interest in the problem derives from utilitarian clinical considerations as well.

Some fifteen years ago, Dr. Alvarez of the Mayo Clinic said that the unequivocal cure of gastrointestinal ulceration awaits a medical procedure based on the complete elimination of acid formation in the stomach, and in order to attain this it is necessary that we learn how to "throw a monkey wrench" into the machinery of the acid-forming cells. This in turn requires an understanding of the cellular mechanism itself, and it is this fundamental cytological process that I am about to discuss—not because I am prepared to offer a monkey wrench for clinical purposes, but because I think we have already gained considerable insight into the machinery we aim to disrupt.

The first suggestion regarding the chemical reaction by which acid is formed in the gastric mucosa was offered by Maly (*24*) in 1877—more than fifty years after Prout (*26*) reported his proof that this acid is HCl. Maly believed that a double decomposition takes place between NaCl and primary sodium phosphate, but he did not state how the two products of the reaction were separated and a reversal of this double decomposition prevented. To compensate for this deficiency, Bergeim (*1*) in 1914 suggested that the NaCl be viewed as reacting with the corresponding calcium acid phosphate, since the resulting secondary phosphate of calcium will precipitate out of solution. Even this mechanism will not bear too close a scrutiny, however. Then, twenty-five years after Maly, Bunge (*3*) suggested that carbonic acid, rather than the H_2PO_4 radical, might be the source of the acidic hydrogen. This possibility remained dormant until 1939, when Davenport (*5*) discovered that the carbonic anhydrase activity of gastric mucosa is enormous—as in the erythrocyte. As a result of this observation, he and Fisher (*7*) revived the Bunge theory and proposed that the entire reaction might be speeded up under the influence of this enzyme—an idea which received considerable support from other investigators. However, quantitative evidence concerning the relative inhibitory actions of thiocyanate ion, sulfanilamide, and thiophene-2-sulfonamide on carbonic anhydrase activity and on the production of gastric HCl *in vivo* proved to be inconsistent with the theory, and in 1946 Davenport was led to retract his hypothesis (*6*)[1]. Instead of H_2CO_3, a number of insoluble weak organic acids have been proposed from time to time as the interactant which liberates HCl from NaCl. These include edestin or some other protein—as suggested by Robertson (*28*) in 1924 on the basis of some very early work of Osborne's; a "lecithalbumin" found in hog mucosa by Rassers (*27*) in 1928; and certain undefined substances which give rise to a phase boundary phenomenon propounded by Clowes (*4*) in 1916, and by Beutner and Caplan (*2*) in 1932.

Many writers have supported a physicochemical reaction based on the Doman equilibrium, but—as I pointed out some years ago (*19*)—this hypothesis demands an impossibly high chloride ion concentration of 7 million molar in the parietal cytoplasm. There is also a process described by A. P. Mathews (*25*) in 1925, whereby intracellular deamination results in the secretion of NH_4Cl by the parietal cell; the salt then undergoes hydrolysis in the neck of the gland, with reabsorption of NH_4OH as ammonia and consequent liberation of HCl into the lumen of the stomach. Our interest in this hypothesis has recently been revived by the excellent work of D. Glick (*8*), wherein he has demonstrated a correlation between the urease activity of minute specimens of the mucosa from successive levels of the gastric gland and the density of parietal cells at these different levels. Since urease is effective in producing ammonia from

[1] In spite of this retraction, the essence of the carbonic anhydrase theory has been retained by several subsequent investigators, e.g.: DAVIES, R. E. and EDELMAN, J. *Biochem. J.*, 1948, **43**, lvii. PATTERSON, W. B. and STETTEN, DEW., JR. *Science*, 1949, **109**, 256.

urea, Glick is now justified in pursuing the hypothesis that this enzyme is an integral part of the intracellular acid-producing mechanism by a process of the kind suggested by Mathews. As another variant of this theory, Hanke (*10*) suggested the cellular extrusion of an organic chloride with a chloroesterase which hydrolyzes the chloride to HCl and a reabsorbable alcohol. Hanke was never able to find such an enzyme in the gastric mucosa and ultimately retracted this hypothesis (*11*).

And finally there is a physicochemical mechanism based on a membrane hydrolysis of NaCl which I proposed some years ago (*12, 19*), and by means of which I attempted to cope with certain aspects of the problem neglected by these other investigators. Before I describe this system, however, I want to tell you some of the accumulated experimental evidence on which this hypothesis now rests.

As one reexamines the various hypotheses concerning acid production by the parietal cell, it becomes evident that they all suffer from two major deficiencies: (1) no physicochemical mechanism is offered to explain how the HCl can be removed from the interior of the cell, which contains buffered cytoplasm at a pH, presumably, in the neighborhood of 7.4; and (2) the presence or absence of other chemical constituents of the parietal secretion is completely neglected. These two considerations are closely interdependent, and in order to include them in any explanation of parietal cell activity it is essential that we know the chemical composition of the pure parietal secretion as it emerges from the cell. To determine this directly in the mammal, by a micropipetting technique like that employed in studying the kidney tubule, is impossible because the inner diameter of the gastric tubule is usually less than 10 microns. Orogastric intubation of unoperated men or dogs is obviously of no avail, because the gastric juice so collected is always contaminated with saliva and the mixed buffer-containing secretions which are regularly regurgitated from the duodenum. Even the gastric juice derived from a Pavlov or other type of gastric pouch, though uncontaminated by these extragastric secretions, is a mixture of several different secretions from the diverse glandular elements of the stomach mucosa itself. In addition to the acid parietal secretion, this mixture comprises: the alkaline mucus from the columnar cells on or near the surface of the mucosa; another alkaline fluid, designated mucoid or dilution secretion, which may originate in the neck chief cells; and finally pepsin secretion from the zymogen or body chief cells. Thus, it is patently impossible to collect pure parietal secretion even from an isolated stomach pouch, not to speak of the intact viscus itself.

And so it became necessary for us to attack the problem indirectly, by studying how the quantitative composition of gastric juice varies with changes in certain pertinent physiological conditions. To describe our experimental procedures very briefly, we prepare our Pavlov pouches in the usual manner from the gastric corpus, as depicted by the original Pavlov diagrams. The Heidenhain pouch is similar to this, except that it is entirely separated from the main stomach and therefore completely deprived of its vagal innervation, whereas the Pavlov pouch continues to receive some of its original vagal innervation through the isthmus.

In the course of our own work (*22*), we discovered that Pavlov and his students had an entirely erroneous idea of the distribution of the gastric vagi in the dog. They believed that only the anterior gastric vagus runs along the lesser curvature, as in man, but that the posterior trunk courses along the greater curvature. Actually, dog and man are alike in that the two trunks run parallel along the lesser curvature. Consequently, in preparing the usual Pavlov pouch, most of the branches of these nerves are cut across and only a small fraction of the preoperative innervation to the mucosa of the pouch area is retained—instead of the full supply as had been generally believed for many years before. As a result, we devised a different type of accessory stomach which avoids transection of the vagal branches in the seromuscular layer and therefore retains practically all of its original supply of parasympathetic innervation (*21*).

Whatever the type of operation, however, the mouth of the pouch is delivered and fixed through a stab wound to the left of the midline, and the skin surrounding the stoma frequently remains uneroded for long periods, provided the dog is dressed meticulously two or three times a day and is generally well cared for otherwise. In the experiment, the animal is supported in a saddle, and a special collecting device is attached to the abdomen with the rubber catheter inserted into the mouth of the pouch. For certain purposes which I shall describe later, the presence of the catheter inside the pouch in undesirable, and so we perform the operation in such a way that the stoma can be kept closed by sphincteric action of the abdominal muscle fibers (*20*). Gastric juice secreted in such a pouch is usually retained for considerable periods of time, even when the animal is left with the dressing on. To draw off the retained fluid, a narrow catheter is inserted, but left there only long enough to aspirate the contents with the aid of a syringe. Using this retention or discontinuous collection technique, the catheter is in contact with the mucosa for only a fraction of a minute, whereas in the older, continuous drainage method, it is held

position throughout the experiment, which usually lasts for several hours.

Now, what is the chemical composition of gastric juice from such accessory stomach pouches? Of course it contains free and combined hydrochloric acid, pepsin, two different mucins, and even small amounts of other organic compounds. Besides these, however, there are significant amounts of other ionic substances—notably sodium, potassium, calcium, bicarbonate, and phosphate. The quantitative composition of pure pouch juice varies extensively, and this variation is particularly marked as regards the acid component. Thus our first efforts aimed to find out whether there was any regularity about these variations, and we started with the fundamental relation between acidity and rate of secretion. Using the continuous collection technique, with the dog in the stand and food as a stimulus, we confirmed the observation of previous investigators that the curves for these two variables, plotted against time of collection after feeding, are roughly parallel (*20*). This was observed with meat alone and with a synthetic nutritionally balanced diet devised years ago by Karr and Cowgill for dog nutrition experiments. This same parallelism was encountered in fasting dogs when histamine was used as the stimulus (Fig. 1—Exp. B 20), provided the dosage was such as to induce a secretory rate of about the same magnitude as in the feeding experiments. More recently we have found this to be true also for other stimuli like meat extract, pilocarpine, mecholyl, and insulin hypoglycemia.

Both Pavlov and Rosemann knew of this correlation between acidity and rate of secretion, but their interpretations of it were radically different (*14*). Rosemann believed that the relation is inherent in the activity of the parietal cell itself, whereas Pavlov believed that the parietal fluid is ejected by the cell at a constant concentration of acid, independent of the rate of secretion, and that all the variations in acidity below this maximum result from neutralization by mucus. Under well-controlled conditions, a healthy nonirritated pouch may be expected to secrete mucus at a fairly steady rate. Hence, when the rate of parietal secretion is high the proportion of admixed mucus will be low and so will the extent of neutralization. When, on the contrary, the rate of acid secretion is relatively low, the proportion of mucus in the mixed juice will be greater and the acidity correspondingly lower. Neither of these investigators had ever been able to confirm Rosemann's theory, nor had any of their subsequent supporters, and so we attempted to obtain crucial evidence on the subject.

To this end we sought changes in the character of the acidity-time curves which might be associated with a marked change in rate of secretion, such as is obtained with maximal dosages of histamine. The results of these studies confirmed Pavlov most beautifully; when the dosage was increased eightfold, the maximum rate of secretion was more than doubled,

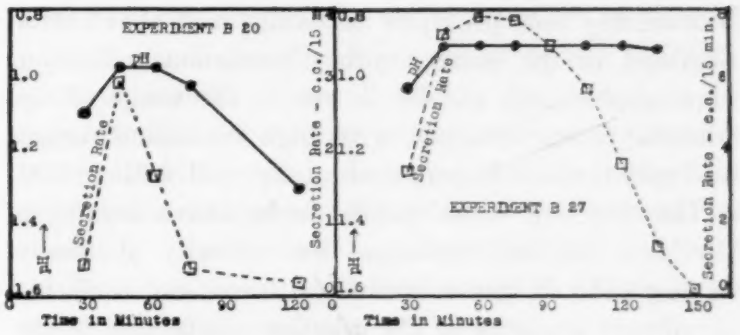

FIG. 1. Continuous collection experiments with histamine as stimulus. Experiment B 20 was performed on a dog with a low secretory rate stimulated by 0.05 mg of histamine per kg; experiment B 27, on a dog with a high secretory rate stimulated by 0.4 mg of histamine per kg.

but the parallelism between rate and acidity disappeared. Instead, the acidity curves rose promptly to plateau values (Fig. 1—Exp. B 27) around pH 0.91 and remained there throughout the experiment, until the rate of flow had again dropped to the postprandial level or lower.

Furthermore, it happens that one of our Pavlov pouch dogs developed a real hypersecretion immediately following parturition—and this did not diminish significantly until her puppies were weaned (*13*). The hypersecretion was probably continuous throughout the 24-hour cycle, and the daily output during the period of lactation was more than ten times that observed during the control periods. A similar though less marked effect was subsequently obtained with another one of our pouch dogs. In spite of this huge increase in secretory activity, however, the total acidity for 66 samples of this hypersecretory pouch juice from the first animal averaged 157 ± 7 mM—corresponding to a pH of 0.89 ± 0.01. The agreement between these data and those from the histamine experiments is striking.

Another confirmation for the Pavlov theory of constant acidity could be obtained if we were able to reduce the flow of mucus secretion markedly in postprandial and similar experiments where the rate of acid secretion is low. Starting with this idea, we reasoned that the mucus may be evoked by mechanical stimulation of the surface epithelium with the collecting catheter, and that additional neutralizing action may sometimes come from a transudate or exudate associated with mild degrees of inflammation or trauma. In order to minimize these two effects, we devised the sphincter-pouch technique that I mentioned previously, for collecting juice without the aid of the collector. Then, when the postprandial and low histamine experiments were repeated by this new tech-

nique, and special attention was given to avoid trauma to the mucosal lining of the pouch, and the pouch was freed of retained mucus by washing out with the first portion of juice secreted in each experiment, we obtained results wholly in accord with our expectation. The acidity rose promptly to values well above those obtained in the corresponding continuous collection experiments, and usually it was in the range of the plateau values obtained with high histamine dosage and spontaneous hypersecretion, i.e., pH 0.91 $\pm$ 0.02.

This limiting value seemed to be characteristic of the pure parietal secretion, but actually specimens having pH's of this magnitude still contain small, but significant amounts of the alkaline component of the gastric juice. To demonstrate this, let us see how some of the other chemical components of this pure gastric secretion are related quantitatively to the acidity. In any one experiment, the time curves for total chloride concentration and titrimetric acidity are parallel, in much the same way that the acidity and volume-rate curves are parallel (*15*). Such variations in total chloride also were in accord with Pavlov's theory, but they directly contradicted the reports of Rosemann and others that the total chloride concentration is constant, regardless of the acidity.

The positive correlation between total chloride and total acidity is even more clearly evident from a statistical analysis of the data obtained in a group of 19 experiments. Plotting these variables directly against each other, we obtained a good straight line (Fig. 2) in the acidity range above 100 mM, irrespective of whether the specimens of pouch juice were collected continuously with the dog in the collecting stand, or intermittently by the sphincter retention technique.

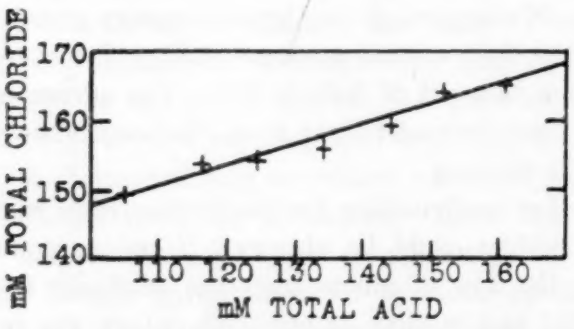

FIG. 2. Total chloride as a function of acidity; mean curve for 121 specimens obtained in 19 continuous collection experiments.

We next calculated the neutral chloride—that is, the combined alkali and alkaline earth chlorides—as the difference between acid and total chloride concentrations, and plotted these values against the total acidity. Again we obtained a straight line (Fig. 3)—of mathematical necessity because of the previous total chloride-acidity relations—but this time the correlation was negative, i.e., the higher the acidity the lower the neutral chloride value. In this particular set of data for 31 specimens of gastric secretion obtained in a series of retention experiments with a single dog, the correlation coefficient is -0.98. This exceedingly high value probably reflects the selected

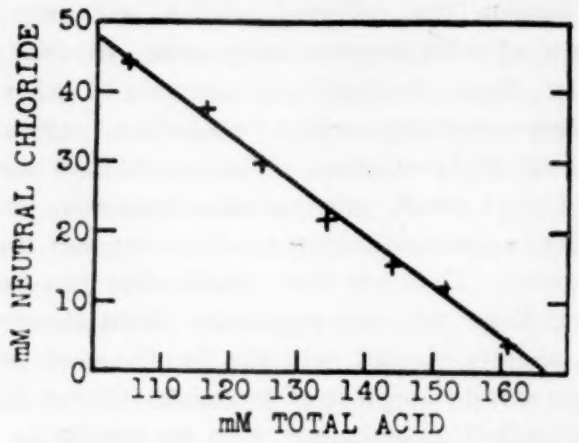

FIG. 3. Neutral chloride as a function of acidity; mean curve for 121 specimens obtained in 19 continuous collection experiments.

character of the specimens, which results from the refinement of experimental technique. The correlation coefficient for the other set of 121 specimens obtained in 19 continuous drainage experiments is somewhat lower, -0.84, but still high enough to demonstrate the validity of this rectilinear relation between neutral chloride and total acidity. Of six such sets of data subjected to correlation analysis, four gave coefficients between 0.97 and 0.99 (*18*). The lowest neutral chloride value actually obtained in these experiments was 3 mM, with a corresponding acidity of 162 mM. The specimen of secretion giving these values was one of the purest we ever encountered, and did not contain a single flake of insoluble mucin.

Now, let us consider the acidity intercept of the graphs for these two variables. This statistic is defined by the point where an extension of the straight line graph crosses the horizontal or acidity axis, and it corresponds to the acidity of a hypothetical specimen of gastric juice which has a neutral chloride concentration of zero. From the data for this particular experiment, the intercept is 167 mM; for all 6 sets of data which we studied statistically, the intercept varied in the range 157–169, with a weighted mean of 165 mM. Similar graphs, of varying statistical reliability, have been obtained for total nitrogen, total solids, ash, and organic solids, but I shall not take time to present the evidence in detail. Suffice it to say that if we accept the validity of this extrapolation technique, the hypothetical pure gastric juice of zero neutral chloride content has the following composition: a total acidity in the neighborhood of 165 to 170 mM; practically the same concentration of free hydrochloric acid, and therefore a combined acidity of zero; a total chloride concentration identical with the total acidity, in con-

formity with the absence of neutral chloride. Everything else is virtually absent—inorganic phosphate, organic phosphorus, both inorganic ash and organic solids, and those several components indicated by the Biuret, Hopkins-Cole, and Molisch tests. The specific gravity is estimated to be 1.001, and the freezing point depression slightly over 0.6° C, very slightly hypertonic (*16*).

I recognize that mathematical extrapolation of physiological data is in general a highly precarious procedure, but since in most of the present instances the experimental data approach close to the intercept values, the range of extrapolation is very short and I believe that we may accept this mathematical technique with considerable confidence. An effort to refute our results has been made by Liu, Yuan, and Lim, by treating the data mathematically in a different way (*23*). However, some of their inferences have already been disproved by Gray and by ourselves, and the remainder still await confirmation. Also, Gray (*9*) has reported evidence to indicate that the parietal secretion is free of sodium but contains potassium at a constant concentration averaging slightly above 7 mM. This is in conflict with our finding of neutral chloride concentrations as low as 3–5 mM in several specimens which we analyzed, and the discrepancy still awaits clarification.

All of the foregoing evidence, therefore, leads to the conclusion that the fluid normally secreted by the parietal cells is a very slightly hypertonic solution of virtually pure HCl, having a titrimetric acidity around 0.17 N and a pH around 0.87. It contains extremely little if any ash or organic solids of any kind, and its composition is practically independent of its rate of formation, the intensity of the stimulus, and probably even the character of the stimulating agent. The combined acidity, neutral chloride, inorganic phosphate, and various organic substances invariably encountered in mixed gastric juice all derive from nonacid buffer-containing secretions: pepsin, mucus, the hypothetical mucoid or dilution secretion, and even small amounts of transudate which enters the pouch from the interstitial spaces. It is this mixture of nonacid buffer-containing fluids that I have designated the "nonacid component" of the gastric juice (*17*).

Now let us return to our main problem of how the acid secretion is made, and particularly how it is separated from the other solutes in the cytoplasm. If the secretion contains only HCl, and that at a nearly isotonic concentration, there is only one answer to this question that I can see (*19*). I believe we must accept the parietal cell as the source of this secretion, if only because of the evidence of Linderstrom-Lang and his associates. Then, somewhere in this cell, there must be a membrane which is permeable to water, hydrogen ion, and the halide ions, but to essentially nothing else. I say halides generically rather than chloride ion alone, because bromide and iodide, when injected into the blood stream as sodium salts, also appear in the gastric juice as HBr and HI in place of part of the HCl. The existence of such a uniquely specific membrane is not difficult to conceive of, in the light of what we already know about semipermeable membranes in general. It may constitute part of the outer cell membrane or the walls of the intracellular canaliculi, which several microscopists believe to be artifacts but which have been accepted by others on reasonably good evidence.

Such a membrane must exist in order for the cell to effect a complete separation of hydrogen ion from all the metal cations. So far as I know, the other theories about HCl formation make no attempt to explain this quantitative separation, although it is one of the most important steps in the process. The several theories that postulate the secretion of a neutral chloride which undergoes hydrolysis *after* it leaves the cell predicate a reabsorption of ammonium hydroxide or of an alcohol of high molecular weight in the neck of the gastric gland, but not of the HCl. If such a differential reabsorption is possible, then I suppose sodium ion and the other cations can also be reabsorbed under these circumstances. But how can the H ion, which possesses the highest ionic mobility known in aqueous solution, not be able to penetrate a membrane at all when the other cations, which possess considerably lower mobilities, are able to penetrate it completely? I think this premise of postsecretory hydrolysis is contrary to all current physicochemical thinking on the subject.

Let us therefore postulate the existence—as part of the parietal cell—of a membrane which is permeable only to water, H ion, and chloride or related ions. Then, consider what happens when some intracellular change takes place which energizes the cell to excrete water (Fig. 4). Some of this water moves out from the cytoplasm, through the cell membrane, and into the interstitial spaces. In so doing it takes with it the by-products of cellular activity and metabolism, along with the usual electrolytes, because the outer membrane of the parietal cell may be expected to resemble that of any other cell in this respect. But some of the water must also be forced out through the specifically permeable membrane of the intracellular canaliculus. In order to keep the osmotic work at a minimum—an invariable thermodynamic requirement in such a situation—the latter water will be accompanied by whatever solute is able to penetrate this membrane, that is H ion and Cl ion. So even though the hydrogen ion concentration of the cytoplasm is ex-

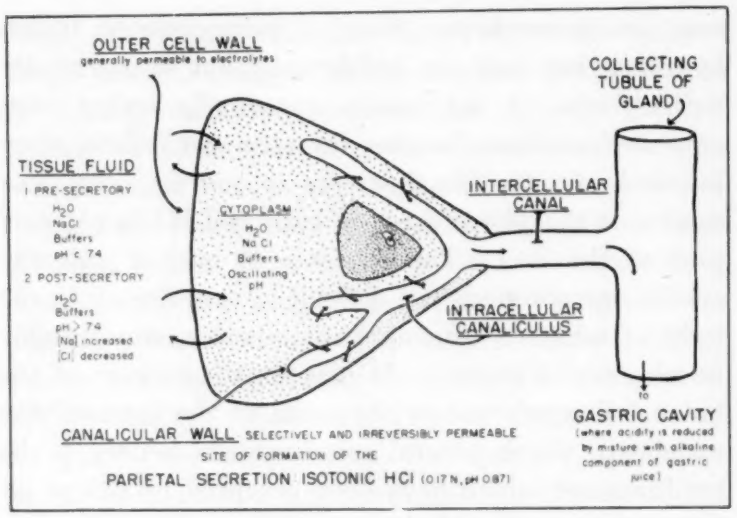

FIG. 4. Schematic representation of the process of HCl formation in the parietal cell of the stomach. The chemical reaction at the wall of the intracellular canaliculus may be written as follows, where B represents the usual cations:

$$BCl + H_2O \longrightarrow HCl + BOH$$

$$BOH + \begin{cases} H_2CO_3 \\ BH_2PO_4 \\ \text{etc.} \end{cases} \longrightarrow H_2O + \begin{cases} BHCO_3 \\ B_2HPO_4 \\ \text{etc.} \end{cases}$$

The over-all energy requirement of the process may be divided into the following components: 1) chemical work (in the cytoplasm and interstitial fluid); 2) electrical work (at the canalicular wall); 3) mechanical work (from the intracellular canaliculus to the open end of the gland tubule); 4) osmotic work (at the canalicular wall). Since the parietal secretion is essentially isotonic with the parietal cytoplasm, the osmotic work is zero.

ceedingly low, some of it will be transported across the membrane, into the lumen of the canaliculus in the form of HCl. Since these ionic movements will leave OH ion and Na ion behind, this reaction is in essence a hydrolysis effected by means of a membrane of highly specialized character, i.e., a membrane hydrolysis.

The HCl solution, passing through the membrane, may be an isotonic or a hypotonic solution, which subsequently undergoes concentration in the collecting tubule of the gland until it reaches osmotic equilibrium with the adjacent interstitial fluid. But the latter process would require two specifically permeable membranes instead of one, and a dual expenditure of energy—first to concentrate the isotonic cytoplasm by removal of a hypotonic HCl solution, and second to concentrate the latter by reabsorption of water. Hence it seems more likely that the HCl will tend to come out of the cell in the first place at a concentration which is isotonic with the parietal cytoplasm, and so keep the osmotic work of this process equal to zero and simultaneously obviate a secondary concentrating process in the collecting tubule. This isotonic concentration of HCl would be about 155 mM, except that probably the intracellular fluid is itself slightly hypertonic with respect to the blood stream, for reasons that I cannot go into here. Hence we may expect to find a value somewhat above 155 mM, which explains the limiting value of 165–170 mM actually found in animal experiments, and the slightly hypertonic freezing point depression. In short, granted the premise of a specifically permeable membrane, the logic of the situation seems to necessitate the secretion of a virtually pure HCl solution, free of all other cytoplasmic components, and possessing a constant concentration somewhat greater than 155 mM.

According to this picture, the acid is formed in or immediately adjacent to the canalicular wall, and its anion derives directly from the sodium and other chlorides of the cytoplasm. The immediate source of H ion appears to be the water, so that the reaction for this process may be written as a membrane hydrolysis of, for example, NaCl to HCl and NaOH. But the instant such alkali is liberated it will be neutralized by the several buffer systems present in cytoplasm. Some of the carbonic acid will shift to base bicarbonate, and a small amount of the latter will shift to carbonate; primary phosphate will be converted to secondary phosphate; and some of the intracellular proteins may be converted to alkali proteinate. The addition of NaOH to these buffer systems may possibly shift the intracellular pH a little to the alkaline side; if so, this will soon be readjusted by the excretion of the alkalinized buffers into the interstitial spaces, along with the products of cell metabolism. The excess NaOH, of course, will ultimately be excreted by the kidneys, after it has been balanced by reabsorption of the HCl in the small intestine.

Thus the membrane hydrolysis theory actually incorporates the theories of Maly, Bergeim, Bunge, and Robertson as essential but subordinate aspects of the over-all process. Even Davenport's carbonic anhydrase may play a role, in spite of his recent negation of it; the discrepancies between the magnitudes of certain inhibitory actions on enzyme activity and acid production may have no significance because the catalyzed formation of carbonic acid is only one of a group of reactions which are secondary to the primary actions of water transport and membrane hydrolysis. How we can bring Glick's ammonia-urease system into the picture, I cannot see as yet, but it will not surprise me at all if this should prove to be one of the mélange of intracellular reactions.

It is noteworthy that there is no evidence for a storage of concentrated neutral chlorides inside the cell. The movement of chloride ion from the blood stream and interstitial fluid to the parietal secretion appears to occur with great rapidity. As judged by the appearance of intravenously injected sodium iodide or radioactive chloride in the gastric juice, reported by other investigators, this entire passage from arteries to lumen of the stomach may require less than two minutes for its initiation. Evidently the cells are not susceptible to fatigue for any significant length of

time. As long as there is an adequate supply of water and chloride, and an adequate removal of waste products, acid production will continue even though other tissues in the body may give evidence of salt depletion. This has been demonstrated by other workers, with animals kept on restricted salt intake for prolonged periods of time.

One might speculate at length about the cellular mechanics and energy requirement of the process of HCl formation, but as yet we have little evidence to work with. Although the osmotic work is zero, energy is certainly being expended in other forms—chemical work for hydrolysis and other reactions within the cytoplasm, electrical for transporting the solution across the canalicular wall, and mechanical for driving fluid out of the cell and through the intercellular canal and tubule of the gland proper. There is already some evidence that the energy can be supplied by glucose, but steps in the conversion are still a mystery. The entire process may be viewed as starting with the movement of water. Then fluid may be forced out because of local changes in hydrostatic pressure, or a pulsatory contraction of some specialized part of the cell, or even an intermittent reversible imbibition of an intracellular macromolecule, but these ideas are all pure fantasy at the present time.

And so I give you a picture of what may be happening in the parietal cell when it manufactures hydrochloric acid. I offer this, however, not as a statement of all that unquestionably occurs, but rather as a hypothesis for further investigation, and one which should be fruitful of many new approaches to the problem. The fact that this mechanism leads of necessity to many of the other theories which have already been advanced, and incorporates them as parts of itself by logical necessity, makes me hopeful of its essential validity. We are probably still far removed from knowing what kind of monkey wrench can be tossed into the parietal machinery for the therapy of ulcer disease, but I hope that this picture of its mechanism will make some contribution toward a simple medical solution of this most vexing clinical problem.

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The Inheritance of Sickle Cell Anemia[1]

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IF A DROP OF BLOOD is collected from each member of a randomly assembled series of American Negroes and sealed under a cover slip with vaseline, to be observed at intervals up to 72 hours, in the case of about 8 percent of the individuals composing the series a high proportion of the erythrocytes will be observed to assume various bizarre oat, sickle, or holly leaf shapes. This ability of the erythrocytes to "sickle," as the phenomenon is commonly described, appears to be attended by no pathological consequences in the majority of these individuals, and they are spoken of as having sicklemia, or the sickle cell trait. However, a certain proportion of the individuals who sickle are the victims of a severe, chronic, hemolytic type of anemia known as sickle cell anemia. This proportion has been variously estimated at between 1:1.4 (*8*) and 1:40 (*4*). The essential difference between sicklemia and sickle cell anemia appears at present to depend at least in part upon the relative ease with which sickling takes place. In sickle cell anemia the erythrocytes may frequently sickle under the conditions encountered in the circulating blood, whereas in sicklemia sickling does not usually occur under these conditions (*12*). This difference has been attributed to a greater tendency of the erythrocytes of sickle cell anemia to sickle when the O_2-tension is reduced, although recently this viewpoint has been challenged (*13*). Perhaps because of this difference—although there may be other factors involved, such as the aniso- and poikilocytosis to be observed in some individuals with the disease, and a greater resistance to hemolysis of trait cells when sickled than sickle cell anemia cells when sickled—the erythrocytes of a patient with sickle cell anemia have a greatly shortened life span, both in the individuals with the disease and in normal persons who have been transfused with the cells of sickle cell anemia patients, whereas sicklemia erythrocytes have an normal life span (*3, 14*).

The ability of the red cells to sickle was observed to have a genetic basis not long after sickle cell anemia was recognized as a clinical entity (*5*). On the basis of a study of one large family, Taliaferro and Huck (*15*) postulated that the ability to sickle was due to a single dominant gene. At that time the clinical distinction between sicklemia and sickle cell anemia had not been clearly drawn, and the inference was that this gene was more strongly expressed in some individuals (sickle cell anemia) than in others (sicklemia). This has remained the accepted hypothesis up to the present time. Several years ago the author, in a review on the clinical detection of the genetic carriers of inherited disease (*9*), was led to suggest an alternative hypothesis—namely, that there existed in Negro populations a gene which in heterozygous condition results in sicklemia, and in homozygous condition in sickle cell anemia. This hypothesis has a counterpart in the relationship which has been demonstrated to exist between thalassemia major and minor (*10, 16*). Recently the opportunity has arisen to give this hypothesis a thorough test.

There exist a number of arguments permitting a critical decision between the two hypotheses. The present preliminary note will consider only one of these arguments. If the homozygous-heterozygous hypothesis is correct, then both the parents of any patient with sickle cell anemia should always sickle (barring the occasional role of mutation; see below). If, on the other hand, the disease is due to a dominant gene with variable expression, only one parent need sickle, although occasionally, due to the chance marriage of two sicklers, both parents may sickle. In calculating the exact proportion of sicklemia to be expected among the parents of individuals with sickle cell anemia according to the dominant hypothesis, certain assumptions must be made. To the best of the author's knowledge, the question of the phenotype of the homozygote has never been raised by those who have accepted the variable dominant hypothesis of sickle cell anemia. For purposes of calculation we shall assume that under the variable dominant hypothesis all homozygotes have sickle cell anemia—alternative assumptions, such as intra-uterine lethality, are possible. We shall further assume that one in fifty heterozygotes also develops sickle cell anemia. Finally, we shall assume on the basis of the clinical data that the fertility of those with sickle cell anemia approximates 20 percent of normal, with the result that only a few individuals with this disease—so few

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that they may be disregarded in so rough a calculation—have one or both parents who are likewise affected. With these assumptions we may calculate, as shown in Table 1, that the proportion of sickling among the parents of individuals with sickle cell anemia should be 0.765. If one assumes that more than one in fifty of the heterozygotes develop sickle cell anemia, or that the homozygote is lethal, then the proportion of sickling parents should be even lower.

Thus far we have tested 42 parents of 29 patients with sickle cell anemia for the occurrence of sickling. In 13 instances both parents were studied and in 16, only one. Tests have been conducted in a variety of ways; especial reliance has been placed on a combination of the techniques described by Scriver and Waugh (*11*) and Hansen-Pruss (*7*), whereby a tourniquet is applied to a finger for 3–5 minutes, and then a drop of static blood from the finger is placed on a slide to which a small amount of Janus green or methylene blue has been added, and it is quickly covered with a cover slip which is sealed with vaseline. Observations are made at intervals up to 72 hours. Five preparations have been made for each individual. Every parent tested to date has sickled. This is the result expected from the homozygous-heterozygous hypothesis outlined above. On the other hand, the probability of the occurrence of such a number of positive parents under the variable dominant hypothesis is $(0.765)^{42}$, or 0.000013.

There are to be found in the literature a number of reports where one or both parents of a child with sickle cell anemia have been tested and found not to sickle (review in reference *9*). The results of tests for the sickling phenomenon are known to be variable; it is felt that the experience quoted may be explained in terms of lack of familiarity with the techniques necessary to elicit sickling.

TABLE 1

CALCULATION* OF THE PROPORTION OF SICKLING TO BE EXPECTED AMONG THE PARENTS OF INDIVIDUALS WITH SICKLE CELL ANEMIA ACCORDING TO THE VARIBLE DOMINANT HYPOTHESIS*

Type of marriage	Frequency of marriage	Frequency of offspring of the indicated genotype			Sickle cell anemia patients	
		Sk Sk	Sk sk	sk sk	Proportion in general population	Proportion among total anemia patients
One sickler parent (Sk sk × sk sk)	$2 \times 0.08 \times 0.92 = 0.1472$	.....	0.0736	0.0736	$0.02 \times 0.0736 = 0.001472$	0.4693
Two sickler parents (Sk sk × Sk sk)	$0.08 \times 0.08 = 0.0064$	0.0016	0.0032	0.0016	$0.0016 + (0.02 \times 0.0032) = 0.001664$	0.5307
Total					0.003136	1.0000

* The assumption is made that all individuals homozygous for the sickling gene (Sk) develop sickle cell anemia, as do 1 in 50 persons heterozygous for the gene, and further that individuals with sickle cell anemia reproduce so infrequently that no significant error is introduced by their omission.

Expected proportion of sickling parents = (proportion of patients having *one* parent sickler) × (proportion of sicklers among these parents) + (proportion of patients having *both* parents sicklers) × (proportion of sicklers among these parents) $= 0.4693 \times \frac{1}{2} + 0.5307 \times 1 = 0.765$.

The approximate frequency of the gene responsible for sickling in the American Negro (p) may be determined from the equation $2p\ (1-p) = 0.08$. Solution of this equation yields a p value of 0.042, from which the incidence at birth of this chronic, disabling, and fatal disease among American Negroes may be placed at $(0.042)^2 = 1.8$ per 1000.[2] The ratio among Negro births in the United States of those with sicklemia to those who will develop sickle cell anemia should therefore be approximately 80 : 1.8 = 44 : 1; in the Negro population as a whole the ratio of sicklemia : sickle cell anemia is significantly higher because of the greater mortality among those with sickle cell anemia. In Africa, the incidence of sickling has been reported to vary from approximately 12 percent in Northern Rhodesia (*1*) to 17 percent in the Gold Coast Negroes and 19 percent in natives of Nigeria and the Camaroons (*6*). This would correspond to a gene frequency of approximately 0.064–0.106, and a frequency of the homozygote of 4.1–11.2/1000. The complex and fascinating problems in gene dynamics raised by frequencies of this order will be dealt with in another paper.

In a genetic situation such as appears to obtain here, where the heterozygote, who may be termed the genetic carrier of the disease, may be readily distin-

[2] The correct formula is $y = \alpha\ p + (1-\alpha)p^2$, where α = the mean coefficient of inbreeding. The value of α for the American Negro is unknown, but probably quite small, in the neighborhood of 0.0005. For present purposes the value of p^2 is a sufficiently close approximation to y.

guished from normal and from the homozygote, it is possible to predict with a high degree of accuracy which marriages should result in homozygous individuals—in this case, children with sickle cell anemia. Since (homozygous) individuals with sickle cell anemia either die young or, if they reach maturity, have a greatly lowered fertility, the vast majority of cases of the disease are the issue of marriages between two (heterozygous) persons with the sickle cell trait. In the absence of marriage between individuals whose erythrocytes exhibit the sickling phenomenon, the frequency of the homozygote would greatly decrease, and sickle cell anemia would tend to disappear, with only a very rare case arising as a result of mutation in a normal individual married to a person homozygous or heterozygous for the sickling gene.

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TECHNICAL PAPERS

New Sectioning Techniques for Light and Electron Microscopy

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The application of the electron microscope to many biological problems has been seriously hampered by the lack of a rapid practical method of cutting uniformly thin sections having adequate area and integrity of structure. Because of the very slight penetrating power of the beam in commercial electron microscopes and the great relative depth of field involved, specimen structure is difficult to interpret when sections are over a fraction of a micron in thickness.

Although various solutions to this problem have been described in the literature, their success and general application have been limited. A departure from classical approaches to the problem has been the high speed microtome (*2*, *3*). However, this precision equipment is not only expensive and complicated but produces a low percentage of usable sections. Moreover, the sections are cut so rapidly and abundantly that selection is time-consuming and uncertain. Several workers (*1*, *5*) have described a technique which uses the thinnest portions of wedge-shaped sections for electron microscopy. Their methods, however, have been laborious and difficult to reproduce.

The most recent effort has been that of Pease and Baker (*4*), who have used standard histological techniques to embed tissue in collodion and paraffin. For sectioning, they altered a Spencer rotary microtome so that the unit of advance was reduced to approximately one-tenth the calibrated value. The microtome was then reported to produce sections as thin as 0.1 μ. Many workers, however, have had trouble in using their technique, mainly because of the exacting demands made on the microtome-advancing mechanism. Another disadvantage has been the difficulty of making very thin sections with the standard embedding media, such as paraffin and collodion.

In recognition of this problem, a promising new development in ultramicrotomy is presented. It consists of a method for obtaining extremely thin sections, involving the use of a methacrylic resin as an embedding medium, a thermal expansion device for advancing the specimen in a commercial microtome, and metallic shadow-casting for increasing observable detail in some of the sections. These techniques form the basis of a new method for producing numerous thin sections suitable for obtaining transmission images at the higher magnifications in the conventional light, phase-contrast, and electron microscopes. Polymerization of *n*-butyl methacrylate provides a rapid and simple means for embedding the fixed biological material in a solid resin. This gives an optically clear matrix from which the sections are cut, one at a time. Smooth, continuous advance of the embedded

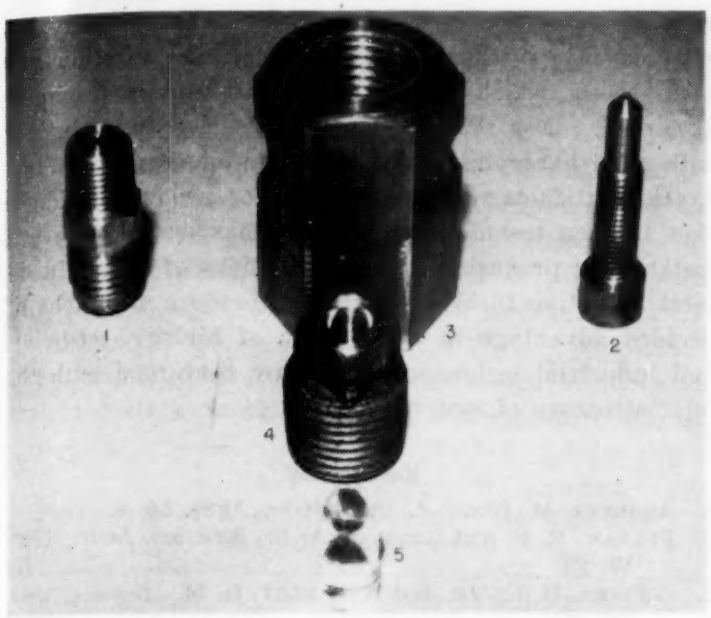

FIG. 1. Disassembled thermal expansion apparatus: (1) and (2) needle valve for carbon dioxide gas, (3) brass expansion chamber tapped for the fittings, (4) standard 3/8-in. brass pipe plug with cavity in face to seat embedded tissue, and (5) tissue in the clear resin with gelatin capsule removed.

specimen toward the knife is effected by the thermal expansion of a brass specimen holder, which permits the Spencer microtome, with its advancing mechanism disengaged, to cut ultrathin sections having uniform thickness, large area, and integrity of tissue structure.

Before being embedded in the polymer, the tissues are fixed and then dehydrated in an ethyl alcohol series by the usual cytological techniques. From absolute alcohol they are transferred to a solution containing equal volumes of absolute alcohol and pure monomeric *n*-butyl methacrylate from which the inhibitor has been removed. After about 1 hr in the alcohol-monomer mixture, the tissues are put in the monomer alone for an equal period. To ensure removal of the alcohol, they are then placed in two additional changes of monomer for at least 1 hr in each.

No. 00 gelatin capsules are convenient embedding molds. The main body of the capsule is set upright in a wooden block or other base and filled with the monomer, to which has been added 1% (by weight) of a catalyst (2,4-dichlorobenzoyl peroxide). After the tissue is placed in the mixture, the capsule lid is slipped on to retard evaporation, and the assembled capsule is placed in an oven kept at a temperature of 45° to 50° C. For even heating, the capsules are suspended by strips of cellophane tape and good air circulation is maintained in the oven.

At the end of 6–8 hr the monomer is polymerized into a solid matrix containing the tissue embedded at the bottom of the clear plastic. An additional period of several hours at this temperature will ensure complete cure. After soaking in water, the gelatin capsule may be peeled from the resin.

An inexpensive device is used for holding and advancing the embedded specimen (Fig. 1). It is essentially a brass block containing a hole threaded at one end to receive a standard $\frac{3}{8}$-in. brass pipe plug. A cavity drilled into the face of the plug provides a seat for the embedded specimen. Behind the plug is a needle valve, which admits compressed carbon dioxide. As the gas undergoes a large change in volume, it cools and contracts the assembly. Stopping or reducing the flow of gas allows the apparatus to approach room temperature again and thus provides continuous advance of the embedded tissue toward the cutting edge.

In practice, the embedded specimen is first cemented into the mounting block with a mixture of pure gum rubber and paraffin. Then, with the device clamped in the jaws of the microtome head, the entire assembly is cooled below room temperature. Upon the appearance of a thin layer of frost on the metal, the knife is adjusted so that the specimen just misses it on the cutting stroke. The specimen is then mechanically advanced at 2- or 3-μ increments until the first slice is made. At this point the mechanical advancing mechanism of the Spencer rotary microtome is disengaged by setting it to zero and the gas flow is reduced or stopped. After a few seconds the specimen can be cut again. Because the specimen is advancing continuously, a quick chopping stroke involving one complete revolution of the hand wheel is necessary. With a little experience one can soon judge the necessary time interval between cuts. Some control of the rate of specimen advance can be obtained by bleeding the carbon dioxide at various reduced rates into the expansion chamber.

Although polybutyl methacrylate has excellent cutting properties, the sections usually are found to be somewhat folded. They are lifted from the knife with a dry camel's hair brush, picked up with a dissecting needle, and placed on a water surface warmed on a hot plate to about 35° C. After a period ranging from a few minutes to an hour or more, many will flatten out on the surface and exhibit bright interference colors. These sec-

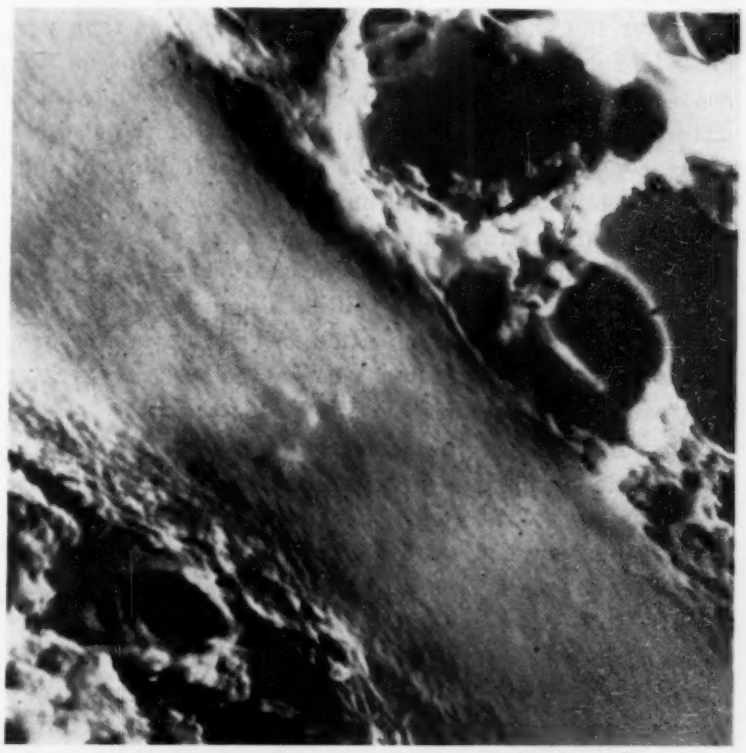

FIG. 2. Frog's eye, section through cartilage, fixed in Bouin's. Electron micrograph, total magnification × 10,400.

tions are then floated onto clean microscope slides and allowed to dry flat. Sections prepared for phase contrast microscopy are placed in acetone or toluene for about ½ hr to remove the matrix and are mounted in Canada balsam. For ordinary light microscopy the matrix is dissolved off and the section stained in the usual way.

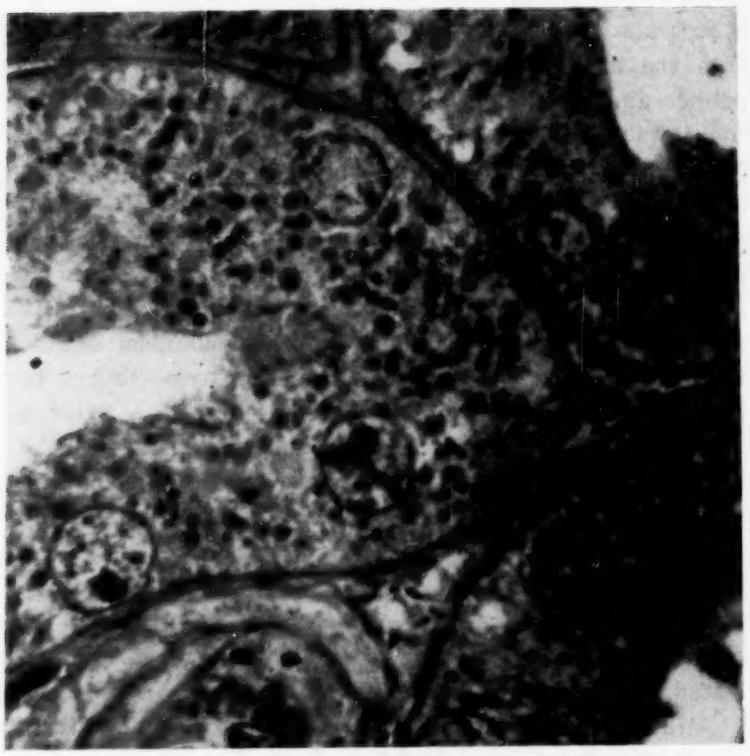

FIG. 3. Dog kidney, tubules and glomerulus, fixed in Zenker-formol. Electron micrograph, total magnification × 1500.

In preparing material for the electron microscope, the sections are floated from the water onto clean glass slides and dried flat. The matrix is then dissolved out by placing the slide in acetone, toluene, or amyl acetate. A dilute solution of collodion in amyl acetate is allowed to flow over the slide bearing the tissue, which is then permitted to dry at room temperature. The collodion film containing the section is floated from the slide onto water, and the specimen-mounting screens of the electron microscope are placed over the area of the film containing the section in the usual manner.

Detection of structural details in some of the tissues was greatly improved by metallic shadow casting of the sections as described by Williams and Wyckoff (*6*). This process, of wide application in electron microscopy, produces a three-dimensional aspect as well as greater contrast in the structural details of the tissue.

The suitability of the sectioning technique for general use was determined by preparing a variety of biological tissues in several different fixatives (Figs. 2 and 3). Although this new method for obtaining very thin sections has given satisfactory results, it possesses certain limitations. Knife sharpness, for example, is of vital importance. However, the tilt of the knife during sectioning is not particularly critical. The greatest chance for failure appears to lie in the polymerization of the embedding mass. Use of low temperature catalysts and maintenance of a curing temperature of 45° to 50° C will usually prevent the formation of insoluble resins. Occasionally tissues are injured during the polymerization reaction and such tissues, which are easily detected, can be promptly discarded. For this reason, the use of fixing solutions with good hardening properties is recommended. While fixation artifacts remain problems for serious consideration, the new technique provides an inexpensive, practical method for producing ultrathin sections of tissue in almost a routine fashion. Such a procedure should be of decided advantage in those fields of biology, medicine, and industrial technology which are concerned with the microstructure of materials.

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Protection of Lupulon and Humulon by Ascorbic Acid

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Lupulon and humulon, two antibiotics of known structure (*3*, *4*) obtainable from hops (*6*), are of special interest because of their activity against *Mycobacterium tuberculosis* (*1*). Both are active *in vitro;* lupulon is active *in vivo* as well. Both show activity against several acid-fast organisms (*1*), against other Gram-positive bacteria (*4*), and against fungi (*2*). They lose activity, in part by oxidation, when boiled in aqueous solutions (*5*).

The possibility of increasing the stability of these substances was investigated for several reasons. It is desirable to have a method by which they can be heat-sterilized in water solution. Also, it has been noted that their antibiotic activity in nutrient media is often temporary, perhaps because of instability at incubation temperature. In brewing, humulon suppresses the growth of certain undesirable bacteria (*6*). However, nearly all of the lupulon and much of the humulon is inactivated during boiling of the beer wort (*7*). If these antibiotics could be adequately stabilized, their effectiveness would increase and their use might be extended to foods.

Humulon and lupulon were prepared as previously described (*2*). For preparation of aqueous solutions, 40 mg of the antibiotic was first dissolved in 1 ml of warm propylene glycol. Phosphate buffer (0.015 M, pH 6.5)

[1] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

was then added to give concentrations of 4 ppm of lupulon and 40 ppm of humulon. Appropriate controls showed that this amount of propylene glycol did not affect the results described below.

These solutions, prepared with and without ascorbic acid, were adjusted to pH 6.5 and heat-sterilized as shown in Table 1. Controls were held at room temperature. An additional set was buffered at pH 8.5 with 0.015 M phosphate, similarly treated, and subsequently adjusted to pH 6.5.

The antibiotic solutions were then added to tubes of nutrient dextrose broth to give lupulon concentrations of 2, 1, 0.5, and 0.25 ppm; and humulon concentrations of 20, 10, 5, and 2.5 ppm. Each tube was then inoculated with one drop of a 1-day-old culture of *Staphylococcus aureus* (A.T.C.C. 6538 P) grown on nutrient dextrose broth. The tubes were examined after 20 hr, and visible turbidity was taken as a criterion of growth.

TABLE 1

PROTECTION OF LUPULON BY ASCORBIC ACID DURING HEAT STERILIZATION

Heat treatment	Concentration in ppm* required for complete inhibition of *Staphylococcus aureus* for 20 hr		
	No ascorbic acid	0.1% ascorbic acid	1.0% ascorbic acid
None	1	1	0.5
Flowing steam, 10 min	>8	2	..
Autoclave, 10 lb, 10 min	>8	1.5	1
Autoclave, 15 lb, 15 min	..	2	..

* Partial (roughly 50%) inhibition was usually obtained by one-fourth of this concentration.

Lupulon solutions containing no ascorbic acid always lost their activity when heated, while those containing ascorbic acid retained a large part of their activity (Table 1). These data represent samples buffered at pH 6.5 but similar results were obtained with the samples sterilized at pH 8.5.

Humulon solutions (buffered at pH 6.5 or 8.5) did not lose their activity when steamed for 10 min or autoclaved at 10 lb pressure for 10 min. Humulon was roughly 10% as active as lupulon against *S. aureus*, giving complete inhibition at 10 ppm and partial inhibition at 5 ppm.

These experiments were run with lupulon and humulon in sufficiently low concentration to permit complete solubility in the buffered solutions. When their concentrations (in pH 6.5 buffer) were increased 10-fold to 40 and 400 ppm, respectively, they were partly in solution and partly colloidally dispersed in the aqueous phase (although a higher solubility has been reported for other humulon preparations [*8*]). Under these conditions the ascorbic acid gave little or no protection. Further attempts to stabilize such dispersions were not made.

For comparison with heat sterilization, small samples of solutions containing 4 ppm of lupulon or 40 ppm of humulon were sterilized by passage through a "UF" fritted glass filter. Both lupulon and humulon solutions lost one-half to three-fourths of their activity during this process, and this loss was not prevented by the addition of ascorbic acid to the solutions.

To determine whether ascorbic acid protects lupulon and humulon during incubation, tubes were prepared containing these antibiotics and ascorbic acid as shown in Table 2. The medium was nutrient dextrose broth (pH 6.5). The ascorbic acid solutions were brought to this pH just before addition to the medium. The tubes were inoculated with *S. aureus* and incubated for 20 days; during this time they were examined at intervals for visible turbidity.

TABLE 2

PROTECTION OF LUPULON AND HUMULON BY ASCORBIC ACID DURING INCUBATION

Antibiotic	Concentration in ppm	Duration in days of complete inhibition of *Staphylococcus aureus*			
		No ascorbic acid	0.01% ascorbic acid	0.05% ascorbic acid	0.25% ascorbic acid
Lupulon	2	1	>20	3*	>20
"	1	1	3	5	>20
"	0.5	0	0	1	5
"	0.25	0	0	0	1
Humulon	20	9	9	>20	>20
"	10	1	1	2	7
"	5	0	0	0.5	1
"	2.5	0	0	0	1

* The reason for this irregularity is unknown.

At each concentration of antibiotic, the time required for growth to appear (or the duration of antibiotic activity) increased with increasing concentration of ascorbic acid (Table 2). Presumably, this relationship was due to protection of lupulon and humulon by ascorbic acid, since ascorbic acid itself did not inhibit growth.

This protective effect is even evident after 1 day. The lowest concentrations of lupulon and humulon to give complete inhibition for 1 day without ascorbic acid were 1 and 10 ppm, respectively. In the presence of 0.25% ascorbic acid these were reduced to 0.25 and 2.5 ppm (Table 2). Without ascorbic acid, part of the antibiotic was apparently inactivated before a significant amount of growth could take place.

Since these antibiotics are known to be readily oxidized (*5*), a tentative explanation of these results is that ascorbic acid has retarded their oxidation.

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An Unusual Lacustrine Delta

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There are several possible approaches to the problem of land form classification. One of the most popular methods involves the gentic classification of forms that will be representative of types actually developed in the rocks and materials of the earth's surface. An attempt is made to establish the particular conditions which operate to produce similar land form types. Nature, however, is a great nonconformist and new types or variations of an old theme are not unusual in the field. Occasionally a particular land form expresses a contrary mood of nature, resulting in a truly unusual set of conditions. It is still possible to classify the type in most cases, but genesis may lie in violation of the usual explanation of origin.

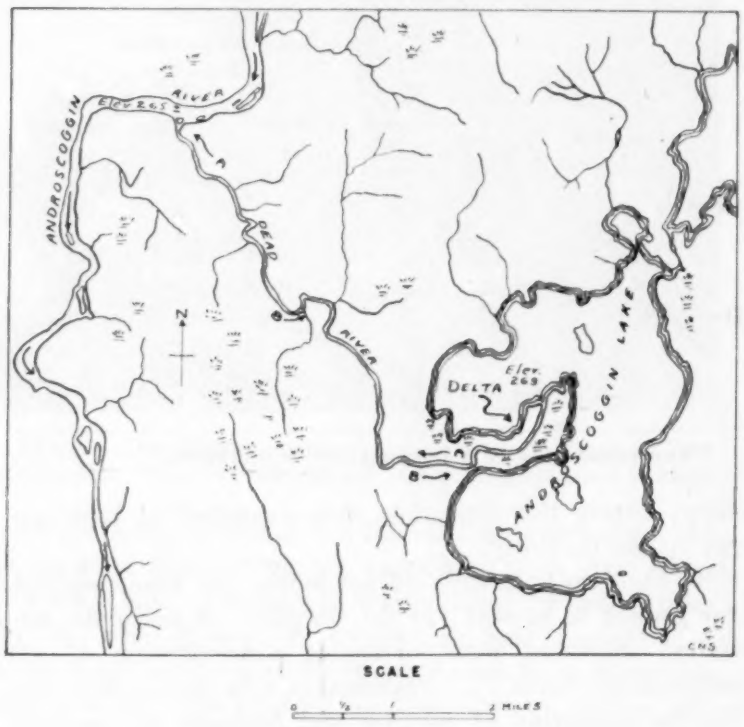

FIG. 1.

One such unique case is found in Androscoggin Lake near Leeds, Maine, about 2 miles southeast of Wayne. A lacustrine delta nearly 1.5 miles long and 0.25 of a mile wide is being built at the head waters of a stream called Dead River. Usually, one expects to find a delta at the mouth or outlet of a stream; at this locality, however, a delta is forming at the opposite end or source of the river.

Throughout much of the year the Androscoggin, one of of the larger rivers in Maine, flows south to the Atlantic Ocean. Dead River, tributary and outlet of Androscoggin Lake, flows northwest and empties into the Androscoggin River. On the sketch (Fig. 1), arrows indicate the direction of flow in the large river and similar arrows marked *A*, show normal direction of flow in Dead River. The difference in elevation between the water in Androscoggin Lake and the Androscoggin River is probably not more than 4–5 ft, and Dead River flows very sluggishly. In time of flood and high water on Androscoggin River, a considerable volume of water moves into the outlet of Dead River and the direction of flow is reversed, as indicated on the sketch map by arrows marked *B*. Water from the larger river then moves into Androscoggin Lake.

Reversal of flow on Dead River occurs generally in the spring months, when runoff is high, and large quantities of sand and silt are being carried by Androscoggin River. This load is obtained partly from the quantities of sand in the region through which the river flows. Surplus water spilling into Dead River—water laden with rock material—moves into the lake, where velocity is checked by the standing lake water and the load is dropped. This deposition normally occurs in similar fashion at the mouths of most streams where deltas are being built. In the case of the lacustrine delta at Androscoggin Lake, however, nature has played one of her pranks and heavy deposition takes place at the head or source of Dead River.

Thermostable Inhibition of Bacterial Hyaluronidases by the Serum of Normal Human Beings[1]

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Hyaluronidase, a mucolytic enzyme, and its substrate hyaluronic acid, a complex polysaccharide which is present in the intercellular substance of connective tissue, compose an enzyme system which appears to be involved in the pathogenesis of various infectious and rheumatic diseases (*2, 6*). Hyaluronidase is elaborated by various bacteria, and is present in spermatic fluid and aqueous extract of testicle. Since hyaluronidases are antigenic, there is current interest in the investigation of hyaluronidase inhibition by the serum of normal human beings and of patients with infectious disease and rheumatic fever. Specific inhibition of pneumococcus hyaluronidase by the immune serum of patients with bacteremic pneumococcus infections has been reported to be thermostable (*7*), whereas the inhibition of testicular hyaluronidase by the serum of normal, nonimmune human beings has been reported to be thermolabile (*1, 3, 4*). In contrast to the thermolability of the inhibitor of testicular hyaluronidase, this report indicates that the inhibitor of pneumococcus hyaluronidase in normal human serum is almost always thermostable, the inhibitor of staphylococcus hyaluronidase is usually thermostable, and the inhibitors of *C. perfringens* and streptococcus hyaluronidases may be either thermolabile or thermostable.

This study investigated the inhibition of similar test strengths of five hyaluronidases by the serum of 50 nor

[1] Supported by a grant from the Robert Gould Research Foundation. This article has been published in abstract (*Fed. Proc.* 1949, **8**, 372).

mal human beings who were between 20 and 40 years of age. Each serum was tested immediately as it was removed from the clot, and again after it had been heated at 56° C for 30 min. The hyaluronidases used in these tests were unrefined filtrates of cultures of type 3 *Pneumococcus*, hemolytic *Staphylococcus aureus*, *Clostridium perfringens*,[1] beta hemolytic *Streptococcus* (group A),[2] and purified bovine testicular hyaluronidase. Twofold serial dilutions of each serum were titrated against the constant strength of each enzyme. Hyaluronidase inhibition was measured by the mucoprotein clot prevention test as previously described (*7*), except that fresh egg albumin was used instead of normal horse serum as the protein component of the substrate. With this modification of the test, a fourfold variation of serum inhibition titer could occur by chance, so that eightfold variation of titer was considered significant. If a 1:3 dilution of a serum did not inhibit an enzyme, inhibitor was considered to be absent from the serum. Hyaluronidase inhibition by serum was considered thermostable if heating the serum caused no significant fall in the titer.

Before heating the sera at 56° C for 30 min, all inhibition of the five hyaluronidases by normal sera occurred in a serum dilution of 1:48 or less, except that two sera in dilution of 1:192 and four sera in dilution of 1:96 inhibited the pneumococcus hyaluronidase, one serum in dilution of 1:384 inhibited the staphylococcus hyaluronidase, and one serum in dilution of 1:96 inhibited the streptococcus hyaluronidase. Results tabulated below reveal thermostable inhibition of the five hyaluronidases by normal sera:

TABLE 1

Hyaluronidase tested	No. of sera tested	No. of inhibiting sera before heating	No. of thermostable inhibiting sera
Pneumococcus	50	47	45
Staphylococcus	49	23	19
Cl. perfringens	50	16	10
Streptococcus	50	12	7
Testicular	48	46	2

Tests upon consecutive daily sera of four persons revealed consistent inhibition of the pneumococcus, staphylococcus, and testicular hyaluronidases. However, there was day-to-day fluctuation in the inhibition of the streptococcus and *Cl. perfringens* hyaluronidases, so that results of the inhibition of these two enzymes tabulated above are of undetermined immunological significance.

A further preliminary investigation of the thermolabile serum inhibition of *Cl. perfringens*, streptococcus, and testicular hyaluronidases was carried out. After this inhibition had been destroyed by heating the sera at 56° C for 30 min, it was completely restored in most sera and partially restored in remaining sera by the addition of complement (*5*). The complement used was 0.5 cc of a 1:30 dilution of normal guinea pig serum, which alone did not inhibit the test strength of the enzymes.

[1] This hyaluronidase was glycerol-dialyzed. It was prepared and supplied by Dr. A. A. Tytell, Cincinnati, Ohio.

[2] Culture supplied by Dr. R. M. Pike, Dallas, Texas.

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Vitamin B_{12} and Cobalt Deficiency in Sheep

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The discovery by Rickes *et al.* (*2*) that vitamin B_{12} is a cobalt complex pointed to the possibility that this vitamin was an intermediary in the metabolism of cobalt in those species requiring this element. It has been suggested by several workers that cobalt, which is known to be required by ruminant but not by nonruminant animals, functions primarily through some unknown mechanism in the rumen, probably related to the microflora. This theory enjoys some but not conclusive experimental support.

At the time that the report of Rickes *et al.* appeared, we were engaged in studies of cobalt deficiency in lambs. Among the symptoms displayed by these lambs were loss of appetite, anemia, loss in body weight, and eventual death. Such symptoms have previously been reviewed by Russell (*3*). Among the treatments under study were the effects of cobalt administration by feeding vs. injection. It was observed that deficient lambs when fed 1 mg of cobalt per day responded quickly in improved appetite, gains in weight, and increase in hemoglobin concentration of the blood. On the other hand, deficient lambs when injected with the same quantity of cobalt showed no detectable response over a period of 7 weeks. It thus seemed possible, assuming that vitamin B_{12} is a necessary metabolite for sheep, that cobalt orally administered may be synthesized into vitamin B_{12} by the rumen flora; and that cobalt injected was incapable of this transformation, possibly because insufficient quantities of it reached the rumen Comar *et al.* (*1*). According to this postulate cobalt deficiency is essentially a vitamin B_{12} deficiency and if this vitamin is furnished the deficient animal directly by injection, a favorable response should follow. Since cobalt salts injected into deficient lambs gave no response it seemed safe to assume that the much smaller amount of cobalt present in vitamin B_{12} would not give a response *per se*.

Cobalt-deficient lambs which had previously been injected with cobalt salts were chosen for this study. All of these lambs had pronounced symptoms of cobalt deciency. They were injected with various amounts of crystalline vitamin B_{12}[1]. The injections were made in-

TABLE 1

HEMOGLOBIN LEVELS OF COBALT-DEFICIENT LAMBS TREATED WITH VITAMIN B_{12}

Sheep No.	B_{12} treatment	Dosage	Hemoglobin level (g/100 ml) Pre-treatment	1st week	2nd week	3rd week	4th week	5th week
		(μg/week)						
1	Crystalline—injection	2	7.1	6.3	died			
17	Crystalline—injection	6	5.2	3.3	2.7	removed		
7	Crystalline—injection	9	4.4	6.0	5.6	5.7	6.0	6.2
9	Crystalline—injection	large*	6.0	4.9	6.0	4.9	4.9	4.9
7	Orally—concentrate	30	6.3	6.2	5.8	6.2†	6.6	6.6
7	Orally—concentrate	120	4.9	4.4	4.2	4.6†	5.7	6.6

* Single injection of 25 μg followed by another of 100 μg during second week.

† Initial dosage doubled.

TABLE 2

AVERAGE DAILY WEIGHT GAIN OF COBALT-DEFICIENT LAMBS TREATED WITH VITAMIN B_{12}

Sheep No.	B_{12} treatment	Dosage	Average daily gain (lb/day) 1st week	2nd week	3rd week	4th week	5th week	6th week
		(μg/week)						
1	Crystalline—injection	2	*	*	died			
17	Crystalline—injected	6	.34	*	*	removed		
7	Crystalline—injected	9	.54	.27	.13	.07	*	*
9	Crystalline—injection	large†	*	.06	.29	.33	*	*
7	Concentrate—orally	30	.03	*	.19‡	.29	*	.36
9	Concentrate—orally	120	*	.23	*‡	*	*	*

* Lost weight for the period.

† Single injection of 25 μg followed by another of 100 μg during second week.

‡ Initial dosage doubled.

tramuscularly twice per week during the period of treatment. Following the period of injections, two lambs were kept under study and fed vitamin B_{12} concentrate.[1]

The levels of vitamin B_{12} chosen for treatment were quite arbitrary, since we had little to guide us from the literature. West (*4*) reported favorable responses in pernicious anemia patients injected with single doses of 3.6 and 150 μg, indicating that the compound had high biological potency.

Results following vitamin B_{12} therapy, in terms of hemoglobin levels and weight gains in lambs, are summarized in Tables 1 and 2. It is noted that there was no significant response in these cobalt-deficient lambs when injected with crystalline vitamin B_{12} in amounts as high as 125 μg. The number of observations was necessarily small since the supply of vitamin B_{12} was very limited; however, the results were clearly negative. Neither was there a response in those lambs fed the vitamin B_{12} concentrate over a period of 6 weeks. Although the concentrate contained cobalt the amount was apparently too small to give a response to cobalt *per se*.

These preliminary and limited observations give no support to the theory that vitamin B_{12} is an important intermediary in cobalt metabolism in lambs.

[1] Supplied through the courtesy of Merck and Company.

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Synthesis of Tris(monofluorophenyl) methane and Tris (parafluorophenyl) methane

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In our search for a liquid dielectric, many aromatic fluorides were prepared in our laboratories, using the Balz-Schiemann (*1*) reaction. These compounds were also coupled by condensation reactions, or by Friedel and Crafts reactions, into symmetrical or unsymmetrical complex molecules.

The preparation of triphenyl methane from benzene and carbon tetrachloride with anhydrous aluminum chloride progressed with such ease and with such good yields (68–84%), that the Friedel and Crafts reaction was used to synthesize substituted triphenyl methane molecules with fluorine in the benzene rings.

Tris(monofluorophenyl) methane was synthesized by reacting 3.5 moles of monofluorobenzene and 1 mole of carbon tetrachloride with anhydrous aluminum chloride according to the procedure for the preparation of triphenyl methane as described and explained by J. F. Norris (*2*). The mechanism of the synthesis accordingly would be:

$$3\ C_6H_5F + CCl_4 + AlCl_3 \longrightarrow (C_6H_4F)_3CCl \cdot AlCl_3 + 3\ HCl$$

$$(C_6H_4F)_3CCl \cdot AlCl_3 + (C_2H_5)_2O \longrightarrow (C_6H_4F)_3CH + CH_3CHO + C_2H_5Cl + AlCl_3$$

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This reaction proceeded smoothly and the product was a liquid rather than a solid like triphenyl methane. The liquid was oily, water-white, and sweet-smelling. In reflected light it exhibited a violet fluorescence. It distilled over in a range of 200–210° C under 125 mm of pressure. The fact that it was a liquid was attributed to the possibility that it might be not a pure compound but a mixture of isomers. Further studies are being made on this point.

p-Difluorobenzene in a similar Friedel and Crafts reaction should form a pure compound and not a mixture of isomers. With the fluorine atoms in the para position in the benzene ring any linkage with the central methane carbon is ortho to one of the fluorine atoms and meta to the other. Since no other linkage is theoretically possible, a pure crystalline compound should result.

Using the same mole concentration as in the previous preparation but substituting *p*-difluorobenzene in the place of monofluorobenzene, the reaction progressed smoothly and gave an over-all yield of 45% of tris(*p*-difluorophenyl)methane. The product obtained was a yellowish crystalline solid. After three recrystallizations from 20 to 30% alcohol, pure white crystals were obtained. The mp of the pure crystals was 98.0–98.5° C.

Analysis (by H. S. Clark, Urbana, Illinois) for $C_{19}H_{10}F_6$:

	Calculated	*Found*	
C	64.78%	64.94%	
H	2.86%	2.86%	
F	32.36%	32.20%	(by difference)

The experimental details for these compounds will be reported shortly.

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Measuring the Thickness of Very Thin Microtome Sections

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Several authors have recently described methods of sectioning biological material for electron microscopy. Some have used a high speed microtome (*3, 4*), others a microtome at ordinary speed with various modifications (*1, 2, 5, 7*). It seems desirable to have sections at least as thin as 0.1 μ, while standard methods result in sections only as thin as 2 μ, or at best 1 μ.

During the war the writer developed a method for cutting some materials consistently at 0.05 μ and even less. Examination of these sections with the electron microscope failed, in the writer's opinion, to show the desired detail in the chromosome material in which he was interested, and so the work was discontinued. The method

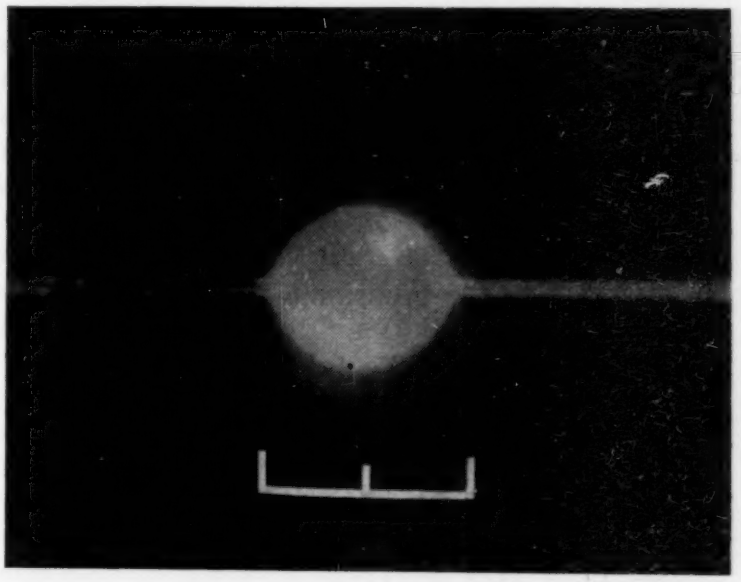

Fig. 1. A sphere of wax resulting from the melting of a section on a thin glass fiber. The length of the scale is 0.1 mm.

consists essentially in the use of: a purified and very hard wax (carnauba) at a low temperature, a specially constructed microtome of the rocker type at ordinary speed, and a beryllium bronze blade. Beryllium bronze is not very hard (about Brinell 380) but its texture is very uniform and it is easy to secure an extremely even and sharp edge. While the method did not succeed in the purpose for which it was intended, it might prove helpful with other materials. The author hopes to publish a full description of it soon. During this work, a simple and accurate means was found for measuring the thickness of the sections. As this may be generally useful, it is described herein.

Several optical methods are available for measuring thin films (*6, 8*), but their use is difficult when the area of the section is only of the order of a square millimeter. A much simpler way is to measure under the microscope the area of the section, then to melt the section into a sphere from whose diameter the volume of wax can be calculated (Fig. 1). To obtain the sphere, the section is caught on a very thin (less than 10 μ) glass fiber, and the fiber is mounted in the field of a binocular. A small loop of electrically heated wire is carefully guided close to the section, which is slowly melted. If the glass fiber is thin enough, an almost perfect single sphere will result; for greater accuracy one may prefer to calculate its volume as an ellipsoid. It is important to watch the heating under fairly high magnification, and to heat slowly, as too high a temperature will cause evaporation.

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Comments and Communications

Ground Substance of the Mesenchyme and Hyaluronidase: A Symposium[1]

The most salient characteristic of this symposium was that of unity underlying a great variety of subjects. For whatever success it achieved was due to the excellent contributions of workers from such diverse fields as chemistry, enzymology, histology, physiology, bacteriology, pharmacology, and clinical medicine. All contributions were fundamentally interested in an enzyme-substrate reaction taking place in the ground substance of the mesenchyme. This was, until recently, an almost abstract conception which now has become a well-defined entity, studied from every angle.

During the last twenty years, catalyzed by the discovery of the spreading factors and important advances in the chemistry of polysaccharides, and through the efforts of many investigators, the newer knowledge of the ground substance has come into prominence. The identification of hyaluronic acid, a component of the ground substance and the substrate upon which some of the spreading factors act, placed a number of these spreading factors in the category of enzymes. In the interim, independent workers studied with excellent results the morphology and physiology of interfibrillar structures. From these contributions, as coordinated in the conference, the ground substance emerged as a coherent unit which, although a part of all organs and tissues, has its own physiognomy and functions; and knowledge thereof appears indispensable for a full understanding of many physiological and pathological phenomena.

The conference was planned and developed along the lines that would seem to be the logical ones to follow in the discussion of a medical subject; that is, it began with chemical and morphological data, passed from this into physiological and pathological subjects, and ended with the clinical applications. Needless to say, this was not the chronological order in which the topics discussed have been studied, since progress in some frequently has been the result of what was already known of the others. As a matter of fact, it was quite revealing in respect to the unity of the symposium that in the great variety of excellent contributions authors rarely kept strictly to specialized fields; cross references were thus numerous and yet discrepancies were minimal.

The first section dealt with fundamental data on the ground substance of the mesenchyme and thoroughly covered the subject. K. Meyer, the chairman, gave a lucid preliminary survey of the mucopolysaccharides of the interfibrillar substance of the mesenchyme. He presented data on the state of hyaluronic and chondroitin sulphuric acids in tissues, and on the physical and chemical properties of these acids. These observations were complemented and extended by the electron microscope studies of Gross, who gave visual representations of the components of the ground substance. Alburn and Williams discussed sources and preparation of hyaluronic acid. A number of histochemical contributions followed. They comprised general studies of the mucopolysaccharides in a great variety of tissues (Bunting, McManus); of the changes in cells and intercellular substances after injection of testicular extract (Bensley); and of comparable changes following inoculation of gonadotropic hormones, and occurring in some pathological conditions such as cancer (Catchpole). It was revealed by these studies that the ground plasm is a dynamic system, changing continuously under the influence of a great variety of factors. In addition, these contributions paved the way for a clearer understanding of the physiological condition existing in the ground substance of the connective tissue. McMaster described it as a viscid medium through which the movement of matter takes place. The section ended with papers dealing with the accumulation of hyaluronic acid in the skin under two extreme conditions of hormone stimulation: in the pretibial edema of Grave's disease (Watson and Pearce); and in the sex skin of monkeys (Duran-Reynals, Bunting, and van Wagenen).

The next section was concerned with the permeability of the ground substance in infection and other conditions. D. H. Sprunt was chairman. The section opened with discussions by Dorfman, Meyer, and Tolksdorf, *et al.* on *in vitro* effects of hyaluronidase. The different modes of action of the enzyme, depending on the linkages of the molecule of the substrate, and the kinetics and specificity of the reactions were reviewed, and the need for establishing a hyaluronidase unit was emphasized. This was followed by a general discussion by Hechter on spreading factors and their mechanism of action, in both the living and the dead animal. The presence of hyaluronidase in inflamed skin was described by Meyer. The importance of bacterial enzymes acting on digestive tract and respiratory mucins (Burnet) was reviewed briefly by Briody. An interesting discussion developed concerning the effect of preparations containing hyaluronidase and other enzymes on the permeability of blood vessels, opposite views being maintained by Zweifach and Chambers on the one side, and by Elster on the other.

With all of these contributions as a background, the conference entered into the important field of the ground substance in infection. A general survey of the subject, covering the effects of mucolytic enzymes, of hydration and dehydration, and of hormones and other agents on the ground substance was given by Sprunt, and Opsahl, White, and Duran-Reynals. This was followed by contributions by Sallman and Birkeland, and by Pike on the production of enzyme and substrate by streptococci, and the meaning of these phenomena in infection. The concept emerged that infection is closely conditioned by a variety of physiological factors which find a direct ex-

[1] Held December 3 and 4, 1948, under the sponsorship of the New York Academy of Sciences. Proceedings will be published in full in *The Annals of the New York Academy of Sciences.*

pression in the permeability of the ground substance. Fluctuations in this permeability result in corresponding fluctuations in the severity of the infectious process. Estrogens and gonadotropic and adrenocortical hormones are especially potent in this connection.

The same general topic on the permeability of the ground substance in infection was continued in the following section. M. Lurie, section chairman, gave an excellent general discussion of the mechanisms affecting spreading in infection, particularly in tuberculosis, in which connection he reported his studies on the paramount influence of constitution, sex, hormones, and still other factors on the disease. After this there followed a series of papers on that puzzling phenomenon of the inhibition of hyaluronidase by blood serum (Dorfman, Hadidian), and on the action of antibodies against streptococcal hyaluronidase (Friou, Quinn), which has proved to be an excellent diagnostic aid in streptococcal infections. The newer theories concerning the possible role played by the streptococcal enzyme or substrate in the pathogenesis of rheumatic disease were discussed. The subject was developed by Ragan and Meyer and discussed by Harris. Facts of great interest emerged in the discussion of this controversial subject—e.g., on the condition of the synovial fluid and the variations in the blood inhibitor for hyaluronidase in rheumatic disease.

In the field of cancer, promising results were reported by Simpson on the influence of hyaluronidase in malignant invasion of tissues, and by Fulton, Marcus and Robinson on an inhibitor for the enzyme found in cancer patients. These studies should be correlated with those of Catchpole, in the first section of the conference, concerning the water solubility of components of the ground substance around malignant growths. Another contribution by Anigstein described curious effects on typhus infection by antiorgan sera.

The last section was devoted to pharmacology and the practical applications of hyaluronidase. J. Seifter, chairman, reported results of his extensive studies on the enzyme, which he found to be pharmacologically nontoxic, and considers the perfect adjuvant, since it enhances the diffusion and thus speeds up the action of a variety of therapeutic agents. Warren and Burket and Gyorgy demonstrated the innocuousness of the enzyme in the case of established infection, in animals and humans respectively. Other authors reported the increased therapeutic effects obtained when hyaluronidase was added to local anesthetics (Kirby, Looby and Elkenoff); to penicillin (Sneierson); and to fluids used in hypodermoclysis (Burket and Gyorgy). The latter authors also reported comparable effects when the enzyme was added to dyes injected subcutaneously for diagnostic purposes.

The beneficial effects of hyaluronidase in some cases of human infertility as reported by Kurzrok offer the only instance of a direct therapeutic effect of the enzyme. To this one could perhaps add its dissolving action on renal calculi, as reported by Simon and Sussman. The effects on fertilization in men and cattle appeared to be a rather controversial subject, as discussed by Sallman and Birkeland and by Chang and Werthessen. The conference ended with these papers.

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Correction

My communication "Note on the Chemistry of Dramamine" (*Science*, 1949, **109**, 574) should have made it clear that the name "Dramamine" applies to the *salt* of β-dimethylaminoethyl benzohydryl ether with 8-clorotheophyllin. As it stands, the first sentence in the second paragraph of my note makes it appear that Dramamine is the ether alone, and that is incorrect.

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Book Reviews

Dentistry in public health. (Prepared for the Dental Health Section of the American Public Health Association.) Walter J. Pelton and Jacob M. Wisan. (Eds.) Philadelphia: W. B. Saunders, 1949. Pp. xi + 363. (Illustrated.) $5.50.

This book presents an unusually fine compendium of dentistry's role in public health. The collaborators have presented good summaries on the topics assigned to them. These summaries, although they are brief, contain the fundamental information which the student or the practitioner needs to develop his thinking in terms of public health service.

The extent of the dental health problem is fully outlined. The great number of people who need dental services, the time it takes to perform these services, and the limited dental man power add greatly to the complexity of the situation and cost of service.

The need for reparative as well as preventive and control service is stressed. Reparative service needs to be thought of as an initial service to care for possible accumulated neglect, and a maintenance service—suggesting regular periodic checkup and care after the initial service has been rendered. This makes the dental health problem a different one from others. The book offers a vast amount of information that should be of special interest to those in the field of dental health.

The role of nutrition and diet is discussed, as well as desirability of laboratory tests to evaluate oral conditions and caries activity. The effect of fluorine in water supply is well presented and indications are that this technique may become an effective agent in the control

of dental caries. The topical application of sodium fluoride to the teeth is also considered.

The need for an effective plan of public dental health education is stressed. The desirability of local, state, and national participation in such a program of service—control and prevention—is well presented. Although now in its infancy, the role of dentistry in the public health program has unusual promise, as judged by the place the contributors to this book ascribe to it.

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Organic syntheses. (Vol. 28.) H. R. Snyder. (Ed.-in-Chief.) New York: John Wiley; London: Chapman & Hall, 1948. Pp. vi + 121. (Illustrated.) $2.50.

The present addition to the series of *Organic syntheses* maintains the high standards set by previous volumes. Directions for preparing the following 37 specific organic compounds are given: 2-acetothienone, 2-acetylfluorene, 9-acetylphenanthrene, 2-allylcyclohexanone, *o*-aminobenzaldehyde, *p*-aminophenyl disulfide, benzoyl disulfide, 9-bromophenanthrene, 4-bromo-*o*-xylene, 3-carbethoxycoumarin, *p*-chloroacetylacetanilide, *m*-chlorophenylmethylcarbinol, *m*-chlorostyrene, 9-cyanophenanthrene, *trans*-1,2-cyclohexanediol, 4,7-dichloroquinoline, 2,5-dihydroxyacetophenone, diisovalerylmethane, 3,4-dimethylaniline, 2,4-dimethylquinoline, 1,4-dinitronaphthalene, diphenylacetonitrile, ethyl azodicarboxylate, ethyl ethoxymethylenemalonate, fluorenone-2-carboxylic acid, hexamethylene chlorohydrin, hydroquinone diacetate, 2-hydroxycinchoninic acid. D,L-isopropylideneglycerol, methyl 4-keto-7-methyloctanoate, 4-nitro-1-naphthylamine, *p*-nitrophenyl sulfide, phenanthrene-9-aldehyde, 1-phenyl-3-amino-5-pyrazolone, α-phenylthiourea, 2,4,7-trinitrofluorenone, vinyl chloroacetate. In each case a check on the preparation has been made by a member of the editorial board and his collaborator. It may be of interest to several readers to record that for their convenience the *Chemical Abstracts* indexing name for each compound is given as a subtitle when that name differs from the title of the preparation. As is the usual practice in this series, the present volume also contains a collective index to material for Volumes XX–XXVIII.

Again, the editorial board should be complimented and congratulated upon its worthwhile project of continuing to make available a repository of miscellaneous syntheses of organic compounds which really work and which will present no unexpected difficulties to the student who needs to prepare one of them. This volume continues the invaluable service of developing good technique. The compounds, in the opinion of the reviewer, have been well selected and the discussions and notes at the end of each preparation are also a real contribution to this all-important field of organic chemistry. Thus, the original purpose of the series is being carried out ably.

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Scientific Book Register

BLOUT, E. R., HOHENSTEIN, W. P., and MARK, H. (Eds.) *Monomers: a collection of data and procedures on the basic materials for the synthesis of fibers, plastics, and rubbers.* (Sect. 1.) New York: Interscience, 1949. 8 chapters. (Illustrated.) $7.50.

CARLETON, H. M., and LEACH, E. H. (Eds.) *Schafer's essentials of histology.* (15th ed.) Philadelphia: Lea & Febiger, 1949. Pp. xii + 655. (Illustrated.) $6.50.

CHALMERS, J. ALAN. *Atmospheric electricity.* New York (11): Oxford Univ. Press; Oxford, Engl.: Clarendon Press, 1949. Pp. 175. (Illustrated.) $3.75.

HILL, FREDERICK C. *Operative surgery.* New York: Oxford Univ. Press, 1949. Pp. xii + 698. (Illustrated.) $12.75.

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NEWS and Notes

Richard H. Young, dean of the College of Medicine of the University of Utah, has been made dean of the Medical School of Northwestern University. Dr. Young succeeds **J. Roscoe Miller,** who has been appointed president of the university.

Stanley D. Wilson, who has just completed twenty years as dean of the College of Science at Yenching University, is retiring this month and will return to the United States during the summer. Dr. Wilson arrived in China in 1917 to organize instruction in chemistry at the Premedical School of Peiping Union Medical College (Rockefeller Foundation), and later became professor of organic chemistry at the College of Science of Yenching University.

William A. Hinton has been appointed clinical professor of bacteriology and immunology at the Harvard Medical School. Dr. Hinton is director of the Laboratory Department of the Boston Dispensary, chief of the Wasserman Laboratory of the Massachusetts Department of Public Health, and special consultant to the U. S. Public Health Service.

C. J. Goodnight, of the Department of Biological Sciences, Purdue University, with his wife and a group of graduate students, left June 23 for Guatemala and southern Mexico to conduct ecological studies on the mammals, birds, reptiles, and animal parasites of tropical mountain communities. The expedition, financed by a grant from the Purdue Research Foundation, will be Dr. and Mrs. Goodnight's fourth research project in these regions. They will continue their work on tropical species of Phalangida in relation to the problem of continental drift.

Carl Voegtlin, former director of cancer research and retired chief of the National Cancer Institute of the U. S. Public Health Service, has been elected honorary member of the Swiss Academy of the Medical Sciences.

Roger Revelle, director of oceanographic research during the Bikini tests and resurvey, has been named associate director of the University of California Scripps Institution of Oceanography.

Isabelle H. Perry has resigned as director of the Department of Oncology of the Women's Medical College, Philadelphia, to accept a position as executive secretary of the Subcommittee on Oncology of the Committee on Pathology for the National Research Council.

Roy G. Hermann has been appointed head of the Biochemistry Section of the Research Laboratories of the Wm. S. Merrell Company, Cincinnati, Ohio.

Visitors to U. S.

Himansu Kumar Mitra, head of the Refractories Engineering Department of Tata Iron and Steel Company in India, visited Pittsburgh June 21–26 as part of a two-month tour of U. S. steel and refractories plants. He came here to attend the meetings of the Rotary International in New York City, to which he was the representative from India, and the Rotary Governors' International Assembly at Lake Placid. On his return trip, Dr. Mitra will spend several weeks in England.

Visitors at the National Bureau of Standards during the week of June 27–July 1 included: **A. J. A. Roux,** principal research officer, South African Council for Scientific and Industrial Research; **M. Escolano,** director, Instituto Tecnico de la Construcción y del Cemento, Madrid, Spain; **Realf Ottesen,** civil engineer, Norwegian Motor Engine Manufacturers Research Laboratory, Trondheim, Norway; **Anand N. Harkauli,** irrigation engineer with Irrigation Department, United Provinces, India; and **Gordon W. Collett,** chief chemist, Wanderlich, Ltd., of Sydney, Australia.

Grants and Awards

Five Bausch and Lomb Science Scholarships, valued at $1,500 each, have been awarded at the University of Rochester. Seven other finalists in the nationwide competition stood so high that they have been awarded comparable scholarships by the university. A total of 61 students, including this year's recipients, have been granted major scholarships at the university as a result of the annual Bausch and Lomb Science Scholarship contests during the last six years. The awards are made on a competitive basis to students throughout the United States.

The Commonwealth Fund has awarded 20 fellowships for 1949–50, among them 12 to the following scientists: *Richard E. D. Bishop,* assistant lecturer in applied mechanics, South East London Technical College, to study mechanical engineering (dynamics and vibrations) at Stanford University; *John Glover,* assistant lecturer in biochemistry, Liverpool, to study the techniques of radioactive and stable isotopes as tracer elements for the investigation of biochemical processes, at the Edward Mallinckrodt Institute of Radiology, St. Louis; *A. Michael Michelson,* research student, Cambridge, to study the broader aspects of nucleic acids and nucleotides at the California Institute of Technology; *John C. S. Paterson,* senior registrar, Department of Medicine, Post-Graduate Medical School of London, to study the chemical pathology of extracellular fluid in the presence of anemia, at the School of Medicine and Dentistry, University of Rochester; *Audrey Jane Pinsent,* research student, Lister Institute, University of London, to study the functions of trace elements in the metabolism of microorganisms, at Stanford University's Hopkins Marine Station, Pacific Grove, California; *William I. Pumphrey,* research leader, Department of Industrial Metallurgy, University of Birmingham, to study the application of metallurgical principles to industry; *Claude A. Rogers,* assistant lecturer in mathematics, University College, London, to study the geom-

etry of numbers and combinational topology at the Institute for Advanced Study, Princeton; *George J. Romanes,* lecturer, Department of Anatomy, University of Edinburgh, to study the structure of the nervous system, especially the ventral horn cells, at the College of Physicians and Surgeons, Columbia University; *George Smith,* extradispensary surgeon, Western Infirmary, and demonstrator in anatomy, University of Glasgow, to study cardiovascular surgery and congenital disorders at the Johns Hopkins Hospital, and at the College of Physicians and Surgeons, Columbia University; *Len C. Taylor,* research student at the Oxford Institute of Experimental Psychology and the Department of Education, Oxford University, to study adolescent educational psychology; *Darcy Walker,* research fellow, Physics Department, University of Birmingham, to study experimental techniques in nuclear physics at Cornell University; and *Peter J. Wheatley,* demonstrator and lecturer in chemistry, University of Oxford, to study the infrared spectra of rapidly burning substances, at the University of Minnesota.

The National Foundation for Infantile Paralysis has allocated almost $2,000,000 of March of Dimes funds for new projects in virus research, professional education and training in poliomyelitis, and study of after-care of the disease.

The grants for virus research were made to New York University-Bellevue Medical Center, University of Minnesota, Michigan Department of Health Laboratories, University of Michigan, Bowman Gray School of Medicine of Wake Forest College, University of Washington, University of California at Berkeley, University of Pittsburgh, Johns Hopkins University, Yale University, Chicago Board of Health, University of Tennessee, and University of Cincinnati. National Foundation headquarters will administer an appropriation of $20,000 to determine the role of flies in the transmission of human polio.

Recipients of appropriations for professional education are American Public Health Association, National Organization of Public Health Nursing, American Physical Therapy Association, Committee on Careers in Nursing, D. T. Watson School of Physical Therapy, Northwestern University Medical School, University of Southern California, Meharry Medical College, and Washington University School of Medicine.

Eight medical schools and hospitals received funds for the study of after-care. They are Children's Hospital, Boston; University of Illinois; Northwestern University; University of California; University of Minnesota; Brown University; University of Vermont; and Cornell University.

Fellowships

The National Research Council announces the availability of a fund of $25,000 from the estate of Charles R. Blakely for support of research in the field of lymphatic leukemia. Application forms for grants-in-aid from this fund and further information may be obtained from the chairman, Division of Medical Sciences, NRC, 2101 Constitution Avenue, N.W., Washington 25, D. C.

The American Cyanamid Company announces the renewal of 15 scholarships for the academic year 1949–50, chiefly in the fields of chemistry and chemical engineering. These scholarships provide $1,500, and are awarded to graduate students in their last year of predoctoral study. Recipients of postdoctoral scholarships receive $3,000.

Scholarships have been established at Brown University, Massachusetts Institute of Technology, Columbia University, Princeton University, University of Virginia, Pennsylvania State College, Cornell University, University of Illinois, University of Notre Dame, University of Michigan, University of Wisconsin, University of Minnesota, University of Colorado, and Purdue University.

The National Foundation for Infantile Paralysis announces the following scholarships and fellowships available in physical medicine, public health, medical social work, and physical therapy, under recently approved appropriations totaling $495,000: $100,000 has been allocated for clinical fellowships in physical medicine for periods of one to three years of study, open to physicians who wish to prepare for eligibility for certification by the American Board of Physical Medicine; $50,000 for fellowships to physicians for one year of postgraduate study leading to the degree of Master of Public Health; $225,000 for physical therapy scholarships in approved schools for men and women in need of financial aid to complete training; $100,000 for medical social work scholarships; $20,000 for fellowships of two to four weeks' duration for the study of poliomyelitis patients at courses to be given at Children's Hospital in Boston, City Hospital in Cleveland, University of Colorado Medical Center in Denver, and Stanford University School of Medicine in San Francisco. All scholarships are on a competitive basis. Further information and application blanks may be obtained from the Division of Professional Education, National Foundation for Infantile Paralysis, 120 Broadway, New York 5, New York.

Colleges and Universities

The Medical College of the University of Vermont has established a Cardiovascular Unit in its Bishop DeGoesbriand Hospital. W. Raab, professor of experimental medicine at the university, will be director and attending physician. Dr. Raab, a former assistant of K. F. Wenckeback, cardiologist at the University of Vienna, has been conducting cardiovascular research at Burlington since 1939.

The Midwest Inter-Library Center, incorporated last March under the auspices of ten Middle Western universities, will be located on the University of Chicago campus. The center, a nonprofit corporation, was established with a $750,000 grant from the Carnegie Corporation and a $250,000 grant from the Rockefeller Corporation. It is a libraries' library and will furnish central housing and servicing for cooperative de-

posit and use of research materials by the participating universities which are: University of Chicago, University of Illinois, Illinois Institute of Technology, Indiana University, State University of Iowa, University of Kansas, Michigan State College, University of Minnesota, Northwestern University, and Purdue University. More than a million volumes will be stored in the six-story building. The site provides sufficient space for a second unit to be built in the future.

Industrial Laboratories

New laboratory facilities for chemical and physical research by the **Kellex Corporation** for the U. S. Atomic Energy Commission in its nuclear reactor development program are now in operation at the Jersey City plant of the M. W. Kellogg Company, the parent company. The laboratory will be headed by W. A. Bain, director of chemical research.

Adenosine-5-phosphoric acid (AMP), the adenine nucleotide usually called muscle adenylic acid, is now available from **Schwarz Laboratories, Inc.** Made by a process that does not require the use of animal tissues, this basic constituent of such vital coenzymes as ATP and coenzyme I has been used clinically in the treatment of cardiac affections, some types of rheumatic conditions, and malnutrition.

Meetings and Elections

Plant and animal nutrition in relation to soil and climatic factors will be the theme of the first of the series of British Commonwealth specialist conferences on agriculture, recommended by the 1946 Official Scientific Conference in London. The conference will be held in Australia in August. Visitors from the U. S., Canada, India, South Africa, and New Zealand will participate.

A colloquium on macromolecules will be conducted on September 2, 3, and 5 by the Macromolecules Section of the International Union of Chemistry, as part of its September 5–11 conference. Subjects under discussion will include the kinematics of polymerization and macromolecules in solution. The colloquium was initiated by H. F. Mark, of the Polytechnic Institute of Brooklyn, and has as its executive committee H. R. Kruyt, chairman; J. J. Hermans, R. Houwink, C. Koningsberger, L. J. Oosterhoff, J. Th. G. Overbeek, and A. J. Staverman, all of the Netherlands. Further information may be obtained by writing to the Executive Committee, P. O. Box 71, Leiden, Holland.

Plans are being made for the **Fifth International Congress of Microbiology** to be held August 17–24, 1950, in Rio de Janeiro. The executive committee includes Henrique Aragao, president; H. C. de Souza-Araujo, first vice president, Genesio Pacheco, second vice president; Olympio da Fonseca, executive secretary; Joaquim Travassos, secretary; Cassio Miranda, treasurer.

The program is built around sections on general and industrial microbiology, medical bacteriology, viruses, Rickettsial diseases, mycology, protozoology, bacterial diseases of plants, microbiology of water and soil, and immunity and resistance.

President Dutra of Brazil has authorized a special round-trip Brazilian boat for the transportation of European members and delegates to the congress, and hotel accommodations will be arranged for them by the committee.

The National Shellfisheries Association, at its annual meeting June 7–9, elected the following officers: president, James Nelson Gowanloch, Louisiana Department of Wildlife and Fisheries; vice president, James B. Engle, U. S. Fish and Wildlife Service; secretary, A. F. Chestnut, University of North Carolina Institute of Fisheries Research; and treasurer, David H. Wallace, Maryland Department of Tidewater Fisheries. The meeting was held jointly with the Oyster Institute of North America and the Oyster Growers and Dealers Association of North America.

An international conference on the optical properties of thin films was held in Marseille, April 19–23. This was one of a series of scientific gatherings sponsored by the French Centre National de Recherche Scientifique. Among the representatives were A. Vasicek from Czechoslovakia; P. Jacquinot, M. Perrot, and P. Rouard from France; K. Greenland, O. S. Heavens, H. Kuhn, and S. Tolansky from England; P. Van Alphen and B. Blaisse from Holland; M. Ballerini from Italy; M. Schaetti from Switzerland; B. Billings, J. Strong, N. Scott, and A. Turner from the United States. The conference was organized by P. Rouard, director of the Physical Laboratory of the Faculté des Sciences in Marseille.

This conference was a manifestation of the current interests in thin film optics. These interests were kindled by the evaporation of metallic reflecting coats by Ritchl and Ornstein, by low index reflection-reducing and high index reflection-enhancing films, and by combinations of all these to produce color films, filters, and interferometers.

The conference itself was outstanding in that each paper presented material completely different from the others. This was particularly remarkable since the publications of several of the members were in journals not accessible to the others. The conference was divided into several sections. The first was devoted to the discussion of the theoretical analysis of the optical behavior of thin films. Several ingenious techniques were presented for the mathematical treatment of films of varying index of refraction. One treatment was based on the electrical impedance analogue, another used a matrix technique in which the equations were set up for many layers, which were then allowed to become infinitesimal in thickness. These treatments would seem to foreshadow the more frequent appearance of such films in the laboratories of experimental physicists.

In the next section most of the papers were on the optical properties of thin films. Two pieces of research reported were new to most of the American group. One was the

demonstration by Professor Rouard of Marseille which showed that the reflection of light going from glass to air can be eliminated by a metal film. In this demonstration light was reflected from a wedge of glass which was coated on the back by a wedge of metal. The metal surface was imaged on the screen and showed a black band at the thickness at which reflection was eliminated. Because of dispersion in certain metals the black band was replaced by a colored fringe. Another piece of new research was the preparation of a film of iron oxide which seemed to combine the mechanical features of TiO_2 film with the desirable optical features of Sb_2S_3. In this same group of papers were a series of talks on narrow band filters. One five-layer filter had both narrower pass bands and higher peak transmission than the older type of silver-dielectric-silver interference filter.

The final part of the conference was given over to the applications of thin films. Although the optical properties of thin films may be a narrow field, this last section showed clearly that it is important in many branches of science. Discussions ranged from the aluminizing of large telescope mirrors to use of interferometers in series for the detection of faint satellites in spectra.

In this section we were especially excited by Professor Tolansky's provocative experiences with interference plates using "Fabry-Perot" reflectivities with "Newton's" spacings. Using his procedures, distances to 10 A or less can be measured. Many unsettled questions were posed by him—for example, he reported unexplained jagged fringes when a thin curved piece of mica is interposed between his plates.

The hosts of the conference organized excursions and diversions which adroitly combined the abundant history and scenery with the scientific activity which is available around Marseille, to produce a lasting impression in the memories of the conferees. All conferees, after a few hours, were fraternizing as if they had been collaborators in the same laboratory.

JOHN STRONG and BRUCE BILLINGS

Deaths

Earle L. Overholser, 60, head of the Department of Horticulture at Virginia Polytechnic Institute, died on April 18. Dr. Overholser was an authority on tree fruits.

Francisco Sierra Soto, Colombian plant scientist trained in the U. S., was assassinated June 19 near his farm on the lower Labrija River in the State of Antioquia. Dr. Soto was engaged in a research and development project conducted cooperatively by the U. S. and Colombia to encourage modern rubber plantations in Colombia.

Ivan L. Nixon, 65, vice president in charge of the Scientific Instrument Division of Bausch and Lomb Optical Company, died June 25. He was a member of the company's board of directors and a director of its Canadian company.

John C. Gifford, 79, professor of tropical forestry at the University of Miami, died June 25 at the Jackson Memorial Hospital in Miami. Dr. Gifford was founder and first editor of the *American Forestry Magazine.*

Harry Manley Goodwin, 79, dean emeritus of the Massachusetts Institute of Technology Graduate School, died June 26 at his summer home at Squam Lake, New Hampshire. Dr. Goodwin's extensive research in physics and electrochemistry included electrochemical studies of the voltaic cell and the electrical properties of inorganic salts at high temperatures.

After a lapse of five years, owing to the war, the **Theobald Smith Award in Medical Sciences,** established in 1936 by Eli Lilly and Company, will again be given at the Annual Meeting of the AAAS. Fellows of the Association should submit names of proposed recipients to Dr. Gordon K. Moe, secretary of Section N, Medical School, University of Michigan, with full information (in triplicate) concerning personality, training, and research work of candidates. Nominations must be received *before September 15.* The award will be $1,000 and a bronze medal, given for "demonstrated research in the field of the medical sciences, taking into consideration independence of thought and originality." An additional amount of $150 is available toward traveling expenses. The recipient must be less than 35 years of age on January 1 of the year in which the award is to be made, and a citizen of the United States.

Past recipients are Robley D. Evans, professor of physics, Massachusetts Institute of Technology, Cambridge; Charles F. Code, Department of Astronomy and Astrophysics, Yerkes Observatory, Williams Bay, Wisconsin; Albert B. Sabin, professor of pediatrics, Children's Hospital Research Foundation, Cincinnati, Ohio; Herald R. Cox, director of virus research, Lederle Laboratories, Inc., Pearl River, New York; and Sidney C. Madden, professor and head of Department of Pathology, Emory University School of Medicine, Atlanta.

The Alabama Science Talent Search for General Gorgas Scholarships, conducted by the Alabama Academy of Science in cooperation with Science Service, has awarded scholarships at the University of Alabama, Tuskegee Institute, Alabama Polytechnic Institute, Birmingham-Southern College, and Howard College. Winners receive four years' tuition and fees from the various colleges and cash awards ranging from $500 to $1,500, given by the Alabama State Chamber of Commerce, in cooperation with business and industry.

The National Registry of Rare Chemicals, 35 West 33rd Street, Chicago 16, Illinois, has submitted the following list of wanted chemicals: Muscone, 1,1-diacetylethylene, colophene, vicine, isoascorbic palmitate, phosphonium chloride, perfluoroheptane, trans-hexahydrophthalic acid, β-tocopherol, isatoic anhydride, isocitric acid, isoaconitic acid, 2-hydroxybenzothiazole, 2-nitrosophenol, adenine thiomethylpentoside, 2,2-difluoroheptane, uric acid riboside, ketosuccinic acid, keracyanin, and muscarine.